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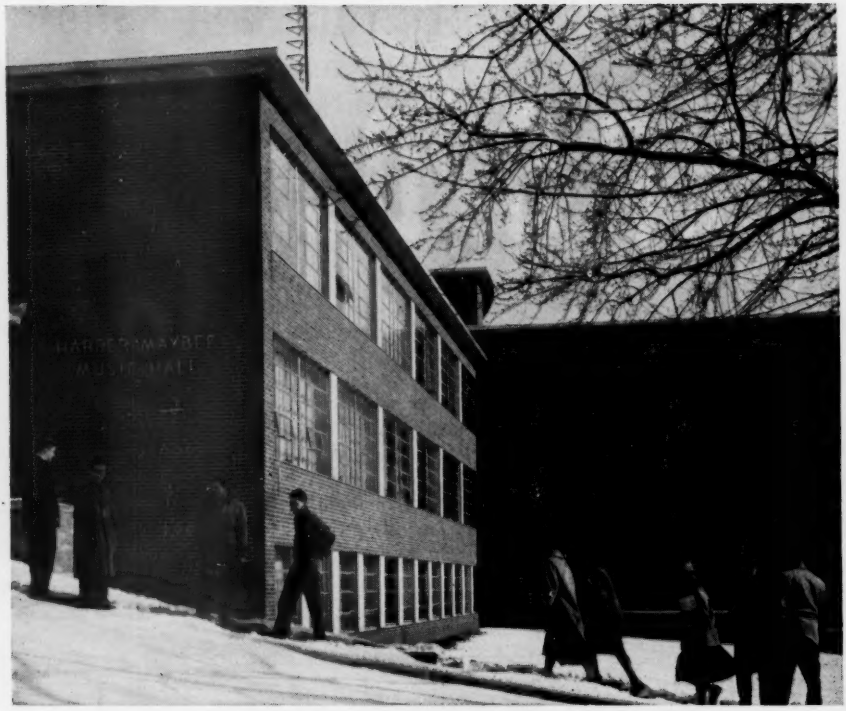

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MICROBIAL PERSISTENCE**

A number of animal species use the defense tactic of "playing dead" when confronted with a hostile threat. It is the purpose of the present discussion to develop the theme that "playing dead" is not limited to the more organized living creatures. For the adaptive plasticity of microbes to environmental influences is such that, in a sense, they too can "play dead" when menaced by an antimicrobial drug.

When a group of patients with a drug-susceptible infection is treated with the appropriate antimicrobial drug, there is a rapid disappearance of all evidences of illness, and the patients are apparently well. In the majority of the patients this apparent absence of infection is permanent. In others, a small subgroup indistinguishable from the rest, the illness reappears after a treatment-free interval. The microorganisms isolated at the time of this reappearance of illness are not drug-resistant in the usual sense, and retreatment with the drug used originally is generally effective. This capacity of drug-susceptible microorganisms to survive drug attack when subsisting in an animal body may be designated as *microbial persistence*.

In the broad sense the term microbial persistence simply indicates something we all know, namely, that microbes have the ability to survive in this perilous world. In the present discussion the term is used in a more narrow sense, however, to designate the survival of microbes in a certain set of definable circumstances. Employed in this way the term microbial persistence describes a phenomenon whereby microorganisms which are drug-susceptible when tested outside the body are nevertheless capable of surviving within the body despite intensive therapy with the appropriate antimicrobial drug. In clinical practice this phenomenon obviously has to do with the post-treatment "carrier state" and with post-treatment relapse. In short, it is this phenomenon which is responsible for our inability to eradicate an infection from a person or a community by the use of drugs.

The initial mention of this phenomenon as something which can be observed in a "group of patients" was intentional. For the clinician does

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* Livingston Farrand Professor of Public Health and Preventive Medicine.

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not usually encounter his patients as a group, all with the same infection. Instead, he sees a few patients with one infection and a few with another. Thus, even though the total number of infections he treats in any one year may be quite large, he might never encounter the phenomenon of post-treatment relapse. This is despite the fact that it occurs with regularity among some of the many who are treated for any particular infection.

A number of us have been fascinated by this phenomenon for slightly more than a decade. During that period my co-workers and I have conducted a number of experimental forays into this field without, I must warn you, penetrating very deeply into an understanding of the phenomenon. Indeed, the best it has been possible to do is to skirt the edges, so to speak. It has been possible to demonstrate: the existence of the phenomenon; its wide occurrence among microbial species; its importance to the clinician and the theorists; and above all, its invulnerability to manipulation with drugs. It has not been possible, however, to obtain more than fragments of experimental data which might lead to its understanding.

It should also be noted that during the eleven years in which our own studies of this subject have been in progress, there have been a number of other groups likewise interested in one or another aspect of the phenomenon. I shall make no attempt in this evening's discussion to present a comprehensive review of all the work which has been done in this broad field. Instead, what follows is to be regarded as a presentation of the development of our own thinking in this field and of certain of the experiments which have formed the basis of that thinking.

At the outset it should be noted that recognition that there is a phenomenon of microbial persistence was not possible before the introduction of penicillin. The reason for this is the fact that the early antimicrobial drugs (quinine, the arsphenamines, atabrine, and the sulfonamides) could never be administered in very large doses because of drug toxicity. As a consequence, there was no reason to postulate any adaptive plasticity of the microbes to explain their survival in the tissues despite chemotherapy. Instead, the survival seemed merely the result of our inability to administer a sufficient quantity of the drug. With the introduction of penicillin into syphilotherapy, however, it soon became clear that inadequate drug dosage was not the principal cause of microbial survival.

Our own interest in this subject originated from experience in the treatment of syphilis. It had long been recognized that syphilis had a remarkable capacity to become a latent infection. Moreover, the conviction was widely prevalent that the time antisyphilitic therapy was most effective was when the early infection was actually producing widespread lesions in the form of generalized cutaneous disease. Treatment given *before* that time

was not quite so effective; treatment given *after* that time was also less effective. Indeed, it is not possible to be certain that once true latency has occurred by natural means, the syphilitic infection is influenced at all by chemotherapy.

With the introduction, first, of short-term intensive arsenotherapy and, second, penicillin therapy, it became clear that even the completed treatment of the highly susceptible early (cutaneous) syphilitic infection was never uniformly successful. Instead, the infection invariably reappeared in a certain percentage of patients (5 to 15 per cent). Although some of these "second attacks" of syphilis presumably represented new infections, some were almost certainly relapses. Moreover, the percentage of incidence of these second attacks was remarkably constant with arsenical or penicillin therapy once certain minimal standards of therapy were attained.

Since penicillin, unlike the arsenicals, was effective against other bacteria, an opportunity was afforded to relate the situation with syphilis to that with other infections. Several possibly pertinent observations were available.

In 1942, Hobby, Meyer, and Chaffee[11] reported that 99 per cent of the cells of a culture of hemolytic streptococci were readily killed *in vitro* by appropriate concentrations of penicillin, but that the remaining one per cent were not uniformly eradicated. In 1944, the late Professor Bigger[8] of Dublin likewise reported that in certain circumstances it was not possible to completely sterilize cultures of penicillin-susceptible staphylococci by prolonged exposure to penicillin. He designated these drug-surviving microorganisms "persisters" in order to differentiate them from bacteria which were drug-resistant.

In 1945, in association with Dr. Edith Lincoln, we had been unable to rid a small group of children of the nasopharyngeal carrier state of type 14 pneumococcus even though the strain was highly susceptible to penicillin. At Fort Bragg, Rammelkamp and Kirby[30] found no sustained changes from penicillin therapy in the pneumococci and streptococci in the pharynx of so-called "carriers." We were particularly interested at the time in the fact that certain of the "carriers" in this Fort Bragg study were patients with the lesions of infectious syphilis.[29] For, despite the fact that the microbes in the nasopharnyx were not permanently affected, the treponemes in the inflammatory lesions of the same patients disappeared rapidly under the influence of the drug.

Observations of this type viewed together with the peculiar drug-parasite phenomena in the treatment of syphilis led to the presentation in 1946[23] of the hypothesis that there was something different about parasites when they were actually producing disease which made them more drug-susceptible and that microorganisms which were not drug-resistant in the usual sense were nonetheless capable of surviving drug therapy in the tissues of man, so that antimicrobial therapy was seldom predictably eradica-

tive. In short, it was postulated that microorganisms in tissues had an important degree of adaptive plasticity irrespective of genotype, and that this plasticity affected their response to drugs.

Attention is thus directed to the state of the parasite. If the most drug-susceptible state occurs when it is producing active disease, this directs attention also to the possible role of the inflammatory lesion, a question

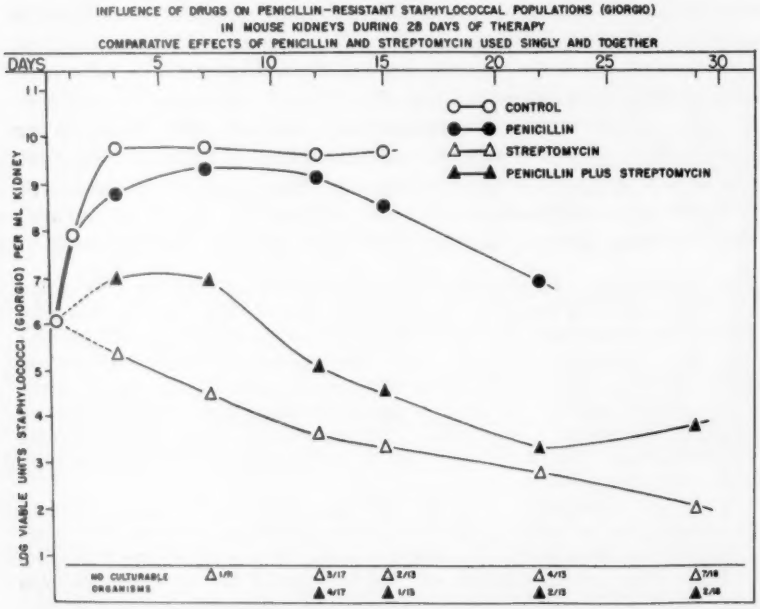

FIG. 1. Reproduced from *Annals of the New York Academy of Sciences*, 1956, *65*, 95.

which will be considered later in this discussion. With introduction of new antimicrobials, additional examples of the failure of a powerful drug to be eradicative in a noninflammatory situation have been observed in clinical practice. The failure of chloramphenicol in typhoid carriers is a case in point, as is the demonstration by Woodward and Smadel[88] that chloramphenicol could be given *too early* in scrub typhus to be completely effective.

Thus the phenomenon of microbial persistence despite appropriate chemotherapy appears to have considerable generality. Moreover, it occurs irrespective of whether the action of a drug, as judged *in vitro*, is bacterio-

static or bactericidal. It is important to note further that at least one and possibly several exceptions exist. Meningococci apparently lack the capacity to persist in the tissues during chemotherapy, and abolition of the "carrier state" with respect to these microbes can be easily accomplished with a sulfonamide. A similar situation probably exists with *Shigella dysenteriae* and the tetracyclines and it may exist with certain antimalarials deemed capable of eliminating the "tissue" phase of *Plasmodium malariae.* Except for these few instances, however, the phenomenon of microbial

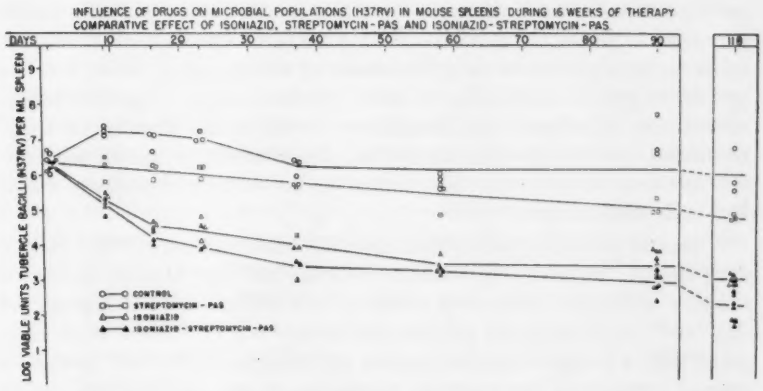

FIG. 2. Reproduced from *American Review of Tuberculosis and Pulmonary Diseases,* 1956, *74,* 102. With the permission of the journal.

persistence seems to occur generally among the microbes which produce infection and disease.

Let us proceed to a demonstration of the phenomenon of microbial persistence in experimental animals. In Figure 1 may be seen the fate of populations of staphylococci in the kidneys of mice with and without the introduction of streptomycin therapy. In the untreated animals, the microbial census rises rapidly until it attains a peak approximating the log of 10. At this point the census stabilizes, and the population remains absolutely constant during the remaining 5 or 6 days until the death of the animal. With the introduction of streptomycin there is no increase in population in this particular organ. Instead, the population slowly falls, but even at the end of the experimental period, in this case 28 days, staphylococci may still be cultured from the kidneys of the animals.

In Figure 2 a similar situation is shown for tubercle bacilli in mice. In this case the spleen is the organ depicted and, as one may see, the census

starts at a level of around 10^6 and slowly rises by more than one logarithmic unit. After approximately 2 weeks at the higher level, the census slowly falls to the original level at which it remains stabilized throughout the experimental period of 118 days. With isoniazid there is an immediate fall in microbial census which then stabilizes at a level of around log 3 and remains constant throughout the remainder of the experimental period. Even at the 118th day it was still possible to culture tubercle bacilli from the splenic tissue of the animals.

What are the possible explanations for this capacity of the microbes to persist in the tissues of the mouse in stable census for many weeks despite presumed exposure to antimicrobial drug? The first thought that comes to mind is the presence of drug resistance of the genotypic form, but this possibility can be very easily excluded. In both of the experiments presented, the culturing of microorganisms in the tissue directly on drug-containing medium showed plainly that the microbes, or rather their immediate descendants, were not resistant to the antimicrobial drugs which had been administered.

A second possibility which deserves only brief mention is the factor of drug-dosage. In similar experiments with isoniazid and tubercle bacilli, for example, a five-fold increase in dosage to 100 milligrams per kilogram per day failed to influence the phenomenon in any way.[20] This is to be compared with a dosage of five milligrams per kilogram which will produce a definite lowering of the microbial population in this experimental system and indeed will produce very marked changes in tuberculous infections in man.

A third possibility which is still frequently stated in the literature is that the drug is confronted by "barriers" in the tissue. These barriers are presumed to exist in the form of "fibrotic walls" of abscesses, large avascular areas, or the "walls" of leukocytes or phagocytes.

The factor of fibrous walls was investigated experimentally by Dr. Robert McCune in our laboratory. Doctor McCune used a technique previously developed whereby triple-layered agar discs about the size of a 25-cent piece are placed in the peritoneal cavity of rabbits or cats.[21] The central layer of the disc can be implanted with microorganisms which in this situation are thus subsisting almost entirely on nutriment provided by the host. In the particular experiment with which we are concerned the discs were not seeded with microorganisms. Instead, Dr. McCune studied the transfer into the center of the disc of a number of commonly used antimicrobial drugs, for example, penicillin and streptomycin. The drug transfer was measured both when the discs had been recently implanted, say, within 12 or 24 hours, and when they had been persisting in the

peritoneal cavities of the animals for a 28-day period. In the latter situation, a 2-millimeter, dense, highly organized wall had formed around the disc. In effect, therefore, each disc represented an area of "dead" tissue, 2.5 centimeters in diameter, surrounded by a living, highly organized, dense fibrous wall.

Dr. McCune was able to demonstrate quite conclusively that the distribution of penicillin and streptomycin and other drugs into the center of the disc occurred rapidly and completely. Moreover, the drug transfer occurred in identical fashion whether the disc had just been implanted in the peritoneal cavity or whether it was surrounded by the fibrous wall at the time the host animal received the drug.*

The question of the barrier provided by the boundary of phagocytes was studied with penicillin and staphylococci by Dr. Ralph Tompsett of the Department of Medicine in our institution.[8] He found that appreciably more staphylococci survived when they were situated within polymorphonuclear leucocytes suspended in plasma *in vitro* than when they were exposed to the penicillin outside the phagocyte. Nevertheless, it is noteworthy that even though the staphylococci survived better within the cells than outside the cells, the populations subsisting inside the cell were reduced by approximately 90 per cent. In other words, there was a great quantitative difference in the results with the intracellular and the extracellular staphylococci, but even the intracellular microbes were influenced by the penicillin to a very large extent.

At the National Institutes of Health, Dr. Harry Eagle[9] has shown that approximately 63 per cent of the penicillin present in an extracellular environment was transferred into Hela cells and mouse fibroblasts. If it can be assumed that human phagocytes behave in the same fashion, very considerable amounts of penicillin should have been present within the phagocytes in Dr. Tompsett's experiments. Nevertheless, in additional experiments he introduced the penicillin in much larger doses, namely 100 units per milliliter. Even in this situation, 5 to 10 per cent of the intracellularly situated staphylococci survived. Thus, the quantitative aspects of the phenomenon were not affected by very considerable increases in the concentrations of penicillin outside the cells.

It appears, therefore, that the ability of a minority of the staphylococci to survive when situated intracellularly is not a consequence of failure in the *delivery* of penicillin to the interior of the cells. On the contrary, the

* A number of observers have demonstrated in caseous lesions in the tuberculous lungs of humans that antimicrobial drugs do indeed penetrate necrotic areas. This has been shown in the case of streptomycin by Canetti[5] and in the case of isoniazid by Dr. Barclay and his associates[1] at the University of Chicago.

persistence of the microbes within the single cell of the phagocyte would seem to be a miniature replica of the situation as it obtains in the host as a whole.

A fourth possibility to explain microbial persistence is that the environment of the inflammatory lesion exerts an antagonistic influence on the action of the antimicrobial drug. This has been a popular concept, and I confess that I have done my share in textbook chapters and similar writings to present it credibly. However, I no longer believe that it can stand up to critical scrutiny as a reasonable explanation.

The case for environmental antagonism of drug activity consists of pointing out that there is a diffusion gradient through nonliving tissue so that, depending on the size of a necrotic area and the drug supply at the periphery, sufficient quantities of drug might not penetrate to the center; that binding to macromolecular protein complexes might easily result in deviation of the drug from its appointed task of reaching the parasite; that changing the environmental pH has a marked effect on the action of certain antimicrobial drugs; and finally, the fact demonstrated in the microbial population studies in mice, that both tubercle bacilli and staphylococci attain considerably higher populations in some organs than in other organs of the same animal. In the case of staphylococci, the organ of maximal census is the kidney; in tubercle bacilli, it is the lung.

Examination of these possibilities for a direct environmental antagonism of drug activity makes it seem unlikely that their role is particularly significant. For example, different drugs presumably have different diffusion gradients through different types of necrotic tissue. But, in the circumstances which obtain in the treatment of humans, it is distinctly improbable that any wide differences materially impede the complete impregnation of the lesion. It will be recalled from the agar-disc experiments, in which only short-term therapy was given, that fairly large-sized areas of nonliving material quickly became full of drug. In the treatment of infections in humans we are not dealing with a period of 12 or 24 hours but with days or months. Throughout these long periods there is a constant delivery of a great excess of antimicrobial drug to the periphery of the necrotic lesion, and, with such a constant excess at the periphery, the drug concentrations in the center should be high.

Essentially the same objection holds against the assumption that drug-effectiveness is seriously impaired by macrocolecular binding. Such protein-binding is an easily reversible phenomenon, and the degree of binding depends upon the concentration of drug in the immediate environment. Hence, even if macromolecular binding were occurring, quantities of drug would also be being released depending on the surrounding concentration.

Here again, when dealing with the large excesses of drug given over the long periods that occur in the treatment of an actual infection, it would seem that however active macromolecular binding might be, it still could not possibly "sop up" all the drug with which the lesion is flooded. The factor of environmental acidity is more subtle and will be discussed in connection with the next possibility.

The phenomenon of "organ difference,"* namely, that certain parasites are more susceptible to certain drugs when subsisting in one organ of an animal than in another organ of the same animal, obviously could represent environmental antagonism of drug activity. An important objection to this interpretation stems from the experiments presented previously dealing with the behavior of populations of staphylococci and of tubercle bacilli in the tissues of the mouse. For example, in the case of the mouse spleen and streptomycin we have an organ in which tubercle bacilli actually multiply during drug therapy at first and never fall below the original census. In contrast, with staphylococci in the spleen, even in the absence of drug therapy, the populations fall steadily, and with streptomycin therapy the populations of staphylococci fall to extremely low levels. Thus we have the same drug, streptomycin which in effect is highly "active" in the spleen when it is acting on staphylococci and considerably less "active" in the spleen when it is acting on tubercle bacilli. This makes it most unlikely that the antagonism of the streptomycin action represents an influence of the splenic environment per se on the drug. It is far more credible to infer that there is something about the respective states of staphylococci and tubercle bacilli when they are subsisting in the spleen which causes a difference in their reaction to streptomycin.

In the same way that the same drug in the same organ is highly effective against one parasite and is considerably less effective against another, one can see that the same drug may act differently in different environmental circumstances *in vitro*. For example, it is well recognized that streptomycin loses a considerable portion of its effectiveness in partially anaerobic environments. This can be easily demonstrated for staphylococci and for tubercle bacilli. Nevertheless, in the very same circumstances, streptomycin is active on *E. coli*.[28] Likewise, pyrazinamide is inactive on tubercle bacilli of human origin when tested in environments within the physiologic range

* It should be pointed out in passing that there is a human counterpart for this "organ difference" in that one could say penicillin is an excellent drug for the treatment of pneumococcal infections of the lung but less effective for the treatment of the same infection when it has penetrated the pleural cavity or exists in the central nervous system. The same holds true with streptomycin and tubercle bacilli, namely, the drug is highly effective in the lung, less so in the pleural cavity or in the central nervous system.[28]

of pH. Yet, when the same environment is made acid, the pyrazinamide is highly active on tubercle bacilli of human origin.[25] This change from "physiologic" pH to acidity does not cause tubercle bacilli of bovine origin to become susceptible to pyrazinamide. Thus in two very closely related tubercle bacilli, so closely related that they almost represent mere strain variants, a change in environment produces drug susceptibility in the one but not in the other. This makes it highly likely that the influence of the acidic environment is on the tubercle bacilli and not on the pyrazinamide.

Now to sum up this question of environmental antagonism of drug activity. It is easy to show that environmental changes result in changes in drug effectiveness. By the same token, all of these changes could equally well represent influence of the environment on the parasite instead of on the drug. Some of the observed changes, moreover, presumably reflect influences which could have been exerted *only* on the parasite. In view of these considerations, together with the fact that a large excess of drug should usually be present in the lesion, it appears that environmental antagonism of drug activity may well occur, but that seldom, if ever, should it attain a magnitude that would provide a satisfactory explanation for the phenomenon of microbial persistence.

Let us turn now to consideration of the possibility that insofar as the tissue environment affects drug-susceptibility it does so by an influence exerted primarily on the parasite rather than on the drug. This makes it necessary to presuppose that the parasite is capable of assuming a state in which it is neither permanently incapacitated by the drug nor does it multiply freely in the presence of drug as do the genetically drug-resistant microbes. In short, the parasite may be said to be "indifferent" to the drug and I have taken the liberty of designating such a supposed state as "drug-indifference." Intimately related to this question are the questions of whether "drug-indifference" may also occur essentially independent of environmental influences and whether such a state could be actually *induced* by the presence of antimicrobial drugs in the tissue environment. As it seems easier from the standpoint of the discussion to take up first the question of the relation of tissue environment to "drug-indifference," consideration of these other two questions will be deferred until later.

We are obviously presupposing that the nature of the environment can produce detectable changes in the parasite and that some of these affect its drug-susceptibility. In order to establish these presuppositions, it is necessary to show that a state of microbial "drug-indifference" exists, that it is reversible, and that it may be induced or specially favored by the particular nature of the tissue environment.

It seems to me that highly credible evidence can be adduced in support of all these presuppositions. Examples exist of the fact that the nature of the environment can change the form of the parasite. R. G. Wittler[39] has observed a considerable morphological change in *H. pertussis* during its sojourn in the mouse lung. In more recent experiments, Wittler, Carey, and Lindberg[40] have observed the reversion of a pleuro-pneumonia-like organism (PPLO) to a corynebacterium during residence in Hela cell tissue cultures. Of even greater interest was the fact that the PPLO form showed little if any cytotoxicity for the cells in which it was situated until yeast extract (or staphylococcal filtrate) was added to the extracellular environment. Considerable destruction of tissue ensued, then the PPLO form was converted to the L-form. The L-form was subsequently converted to the orthodox corynebacterium on further cultivation with the aid of mucin. These investigators state: "From our observations, it seems likely that factors which produce changes in host cells may in turn result in changes in the form or in the type of growth of the infecting organism, yet this may not always be readily apparent when the organism is again cultured *in vitro*."

A particularly dramatic example of this same type of phenomenon was recently observed by Dr. Dubos in his studies with *M. fortuitum*.[7] This mycobacterium, like tubercle bacilli and staphylococci, proliferates more readily in certain organs of the mouse than in other organs. For example, the peak census of this microbe is attained in the kidney rather than in the lung. Dr. Dubos has observed that cultures of *M. fortuitum* assume either one of two quite distinct forms. In one form the colonies appear as a hard heaped-up button without a peripheral lacy skirt; in the other form, the colonies are flatter and extend for a considerable distance from the central core forming a peripheral "skirt." The important thing is that the microbial populations cultivated from one organ of the mouse assume one of these colonial patterns, whereas when cultivated *on the same medium* but from another organ they assume the other colonial pattern. There is every reason to believe, therefore, that depending upon certain aspects of the environment in the particular organ, the assumption of one or the other of these colonial patterns becomes predominant. Thus the environment of the organ appears to determine the state of this particular parasite. Dr. Dubos has carried these studies even further and can now demonstrate that a purified substance known to be present in the tissues, when incorporated into the medium, can determine which of the forms is assumed by the parasite.

From these examples chosen—the studies of Wittler and associates and those of Dubos—it may be seen that the first presupposition that environ-

mental factors can determine which of several possible different states will be assumed by a parasite is clearly the case. The presupposition that there should be a state of "drug indifference" and that it should be reversible can very easily be demonstrated by a simple experiment with penicillin and Group A beta streptococci in a favorable medium, maintained first at ice-box temperature and subsequently at incubator temperature. In the cold environment the microbial population remains absolutely unchanged, even though it is afforded a favorable medium and high concentrations of penicillin are present. When this same culture is removed from the cold environment and put in the warm environment, two things happen at the same time. The population rapidly increases in the absence of penicillin and rapidly falls in the presence of penicillin. This simple experiment, which has been performed with a number of microbial species and a number of drugs, clearly shows that there is a state of "drug-indifference," that it is reversible depending on environmental changes, and that such states can be induced or favored by the nature of the environment.

Admittedly, the particular environment chosen from the demonstration cited is a highly artificial one. What is the situation in circumstances more directly related to the circumstances of an infection? First, let us start again with the simple system of Group A beta hemolytic streptococci and penicillin. Virtually all of the published experiments on the dynamics of the action of penicillin or other drugs *in vitro* have employed a relatively standardized system. In this system actively growing microbial populations with a total census of about 10^8 are subjected to the action of the drug, let us say, penicillin. In these circumstances there is a rapid and essentially straightline fall (on a logarithmic scale) of the microbial population to low levels or indeed, in many cases, to zero within a few hours. It is the fact that this does not happen in the animal body that represents the phenomenon of microbial persistence. But why has there been this disparity between the events in the test tube and what happens in the body?

In some unpublished experiments in our laboratory conducted by Dr. Carl Berntsen it was noted that this rapid fall in microbial population under penicillin *did not occur* when the initial populations of streptococci were high, say around 10^8 or 10^9. In the latter circumstances an impressive portion of the population of supposedly penicillin-susceptible streptococci was nevertheless capable of withstanding the action of penicillin for a week or longer. The census slowly fell, but even at the end of the week appreciable numbers of streptococci could be cultured from the medium. In short, penicillin acted dramatically on small microbial populations of the order of 10^6, but its effectiveness was reduced to a very great extent on larger populations of the order of 10^8 or 10^9.

The question immediately arose as to how great are the microbial populations in the lesion of an infection? Dr. Berntsen made a number of studies of this point using vegetations from heart valves of untreated patients dying of bacterial endocarditis (medical examiner's cases), freshly excised tonsillar tissue handled with techniques designed to prevent microbial proliferation of the microbe after excision, and vegetations from experimentally produced endocarditis in rabbits. In addition, there was in progress in the laboratory at the same time a large number of quantitative studies with the microbial enumeration technique of the infections produced in mice by tubercle bacilli and staphylococci.

All of these observations tended to show the same thing, namely, that in the mouse organ or tissue with the greatest microbial census (or in the other tissues of man mentioned), the value for the microbial population almost always ranged between 10^8 or 10^9 per milliliter of emulsified tissue. A minor exception to this is the fact that in some circumstances in the lung the peak census of tubercle bacilli ranges between 10^7 and 10^8. It is of considerable interest that these values of 10^8 and 10^9 for the peak census of these various microbes are essentially the same as are attained by these three microbial species when they are allowed to grow under favorable conditions in the test tube. Thus with staphylococci, beta hemolytic streptococci, and tubercle bacilli, both in the test tube and in the organ of the mouse in which the highest population occurs, the peak value is essentially the same, namely, 10^8 or 10^9.

Populations of penicillin-susceptible streptococci or penicillin-susceptible staphylococci of this size, 10^8 or 10^9, are not very markedly influenced *in vitro* by the introduction of penicillin. In short, *in vitro* at least, they are "drug indifferent"; they are not "drug resistant." Moreover, in the animal, populations of this size of either streptococci, staphylococci, or tubercle bacilli are considerably less affected by isoniazid or penicillin than populations around 10^6 in the same animal.

We have, therefore, to ask ourselves the question: in what ways other than in size do these large "drug-indifferent" microbial populations differ from the smaller drug-susceptible populations?

With the large populations the microorganisms have been growing in the environment for a longer period in order to attain the high census. This means two things: the large population is an older one, and it has been reacting with its environment for a longer period than is the case with the small populations. Thus there has been a greater opportunity for chemical modification of the environment, either in the culture medium *in vitro* or in the inflammatory lesion in the animals.

In a further series of unpublished experiments by Dr. Carl Berntsen it was shown that when age of the population and the environmental alterations produced by growth are divorced experimentally, large populations of streptococci are as susceptible to penicillin as are small populations. In these experiments, large (10^8 and 10^9), populations of Group A beta hemolytic streptococci were employed in which the majority of the microbes were less than 4 hours old. Such high populations of very young organ-

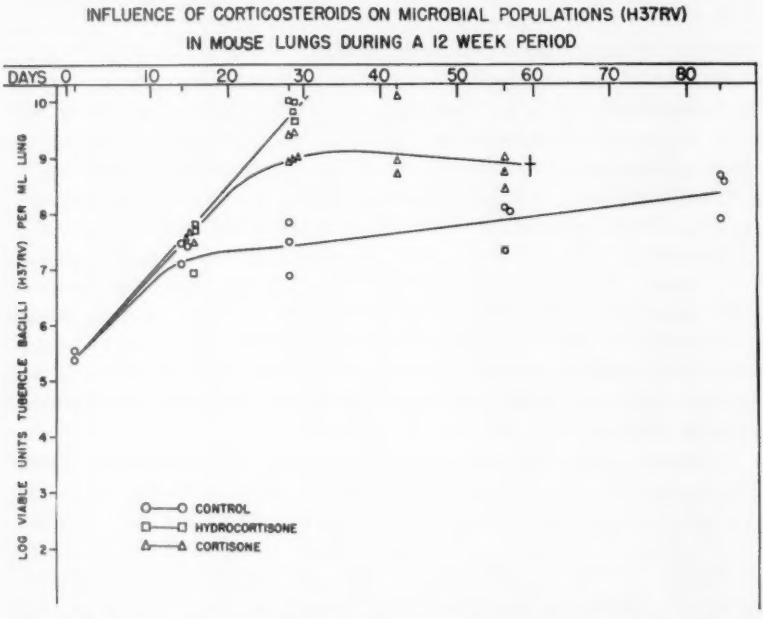

FIG. 3. Reproduced from the *British Journal of Experimental Pathology*, 1957, *38*, 419.

isms were obtained by cultivating the streptococci on a large scale and resuspending them in an appropriate volume of fresh medium. It was possible to demonstrate that when the majority of the cells in such a large population were less than 4 hours old, the population was just as susceptible to penicillin as when the young cells were initially present as a small population. It was also possible to show that this penicillin-susceptibility of the large 4-hour-old populations obtained to the same extent when the populations were implanted in the mouse muscle as when they were inoculated into a test tube. Thus it may be seen that not only were these

large drug-susceptible populations quite young, but in both the mouse and in the test tube they were being tested in an environment which they had had no previous *chance to alter.*

If the relative insusceptibility to penicillin of the large "naturally" grown populations of streptococci were reversible by changing the environment, it would constitute evidence that the "drug-indifference" of the microbes represented an adaptive state to certain environments. Indeed, one aspect of the factor of aging of the population itself conceivably could be altered by environmental changes and regarded as an adaptation, for the proportion of young microbial cells to old cells, i.e., the capacity of the population to form new cells, might vary with the environment.

A related aspect of this question of reversibility of the "drug-indifference" of large populations is the fact that if the peak census attained in the mouse tissues or in the test tube *were not the maximal attainable census* in these situations, it follows that what we designate as the peak census in actuality represents a microbial population under some inhibitory influence. In both the mouse and the test tube it can be shown that this is the case, and that the peak census obtained by the microbes is not the maximal obtainable census if the circumstances are modified.

In the mouse, this can be demonstrated by the administration of cortisone to animals infected with tubercle bacilli, as may be seen in Figure 3. The lowest curve in the figure shows the population of tubercle bacilli in the mouse lung attaining a peak between 10^7 and 10^8 and then stabilizing at essentially that census thereafter. In the animals maintained on hydrocortisone, however, the microbial census was as high as 10^{10}, yet the animals were able to survive at least for a period of a week or so in these circumstances.* In the test tube with staphylococci and penicillin, Dr. David Rogers[81] of our Department of Medicine has shown that a microbial population which has become stable at a census of 10^8 will increase to 10^9 simply by the process of shaking which, of course, also involves aeration.

These two sets of observations are interpreted as showing that the environment constituted by the tissue or the test tube in which microbial populations have grown to 10^8 is exerting an inhibitory influence at that point, for if the cessation of population increase were determined by factors within the parasite and independent of the environment, it should not be regularly influenced by environmental change. Coincidental with this inhibitory influence of the environment on the microbes is their relative indifference to the drugs.

* It should be noted that there is a definite tendency toward stabilization even of these very large microbial populations developing in hormone-influenced animals.

It can easily be shown in the test tube that the inhibitory influence which causes the growing populations to stabilize is not a question of the exhaustion of nutriment from the medium. In Dr. Berntsen's experiments fresh daily inocula of streptococci were repeatedly allowed to grow day after day to the point of stabilization in the same medium from which the bacteria were removed by filtering at the end of each 24-hour period. After 8 or 10 days, the fresh inocula of streptococci in this same medium would still grow logarithmically up to the same peak census and then stabilize. Thus nutriment, in the sense of something generally available through the medium, was not exhausted under these conditions. Likewise, to some extent, the experimental results stand against the concept that toxic substances produced by the microbes are responsible for the cessation of population increase. Certainly the possibility is excluded that a filter-passing substance was present in sufficient quantity to exert an influence throughout the medium as a whole. The possibility remains, however, that minute concentrations of inhibitory substances might be elaborated in the immediate environment of the bacteria or might adhere to the filter. Thus, it appears that there is some factor or factors associated with attainment of the "crowded" state by the microbes which exerts an inhibitory influence on further population growth. This factor may be something intimately associated with the immediate environment of the microbe but does not seem to be something which is detectable throughout the environment as a whole.

To recapitulate, it can be shown that a population of microbes growing either in the tissues or in the test tube becomes subject to some inhibitory influence before it reaches the maximal attainable population in those circumstances. And, at the time that the population becomes subject to the inhibitory stabilizing influence, the microbes also become "drug indifferent."

The important thing from the standpoint of the argument is the fact that when this environment which has now become inhibitory is altered, not only do the populations show themselves capable of further growth, but they also once again become *drug susceptible.* A small aliquot of the large "drug indifferent" microbial population is quite susceptible to penicillin when tested separately in fresh medium. Moreover, Dr. Rogers has clearly shown with the penicillin-staphylococcus system *in vitro* that penicillin-susceptibility reappears coincidentally with the new population upsurge produced by shaking and aeration.

It appears, therefore, that it is not the age of the population per se, but its adaptive changes to the environment it has altered while attaining that age which are associated with its "drug-indifference." These environmental relationships (i.e., the consequences of crowding) are responsible for the failure of the population to continue to increase in the test tube and con-

ceivably are responsible in part for the failure to continue to increase in the tissues. In terms of the population as a whole, age is a factor, but it is a factor which remains responsive to environmental change. When the environment is altered so that the same "old" cells begin to divide into a greater number of freshly born "new" ones (to the extent this occurs), the age of the population has been made more youthful.*

In considering this question of "age," it is intriguing to note that if microbes possessed the capacity to be susceptible to penicillin or isoniazid only when they were quite young, e.g. less than four hours old, certain of the experimental observations presented above or subsequently would be satisfactorily explained.

At first glance, this explanation appears to be identical with the familiar one that "penicillin acts only on multiplying bacteria." In crude terms the two explanations are indeed the same; nevertheless, a subtle difference does exist between them. With the familiar explanation it is implicit that microbial cells in the metabolic state which leads directly to cell division are *ipso facto* in a state in which they can be "killed" by penicillin in the environment. At any time a so-called "resting cell" enters into this pre-divisional state it is drug-susceptible. With the presently proposed explanation, multiplication or cell division is only a side aspect of the issue. Instead, it is postulated that as young microbes grow, they lose something or gain something which makes them resistant to drugs.** In other words, an adaptive mechanism becomes activated, and the capacity thus developed might well remain irreversible in terms of the individual cell. When the environment is suitably altered so that more of these "old-adapted" cells give birth to young "unadapted" cells—to the extent this occurs—the population becomes drug-susceptible.

In short, the environment tends to antagonize drug-effectiveness by becoming unfavorable for the birth of drug-susceptible microbes.

Let us turn to consideration of that other aspect of the aging of a parasite-host interaction, namely, the inflammatory changes produced in the environment. Dr. Harry Eagle[8] has studied the influence of penicillin on Group A beta hemolytic streptococci which have been introduced into the gastrocnemius muscle of the intact mouse. He was able to show that when the administration of penicillin was withheld for 18 or 24 hours after the start of the infection, it had far less of a population-reducing effect than

* The concept that bacterial cultures exhibit a period of "physiological youth" with changes in morphology and behavior resembling what occurs in more highly differentiated multicellular organisms has been suggested by several writers (e.g. Sherman and Albus, 1924) and is presented on page 143 of *The Bacterial Cell* by R. J. Dubos (Harvard University Press, 1945).

** Recent work (see Park and Strominger[27]; Lederberg[26]) suggests that penicillin interferes with the synthesis of the cell wall of young microbial cells and that this does not occur in fully developed cells.

when the drug was administered within a few hours. This observation has been repeatedly confirmed in a number of laboratories including our own. It gives rise at once to the question under present consideration, namely, did the inflammatory environment in the older infection exert its influence on the drug or on the parasite?

There are two sets of observations which indicate strongly that the effect of the environment was mediated directly on the parasite and that there were no important environmental effects on the drug. In recent experiments in our laboratory by Drs. McCune and Dineen it was found that when staphylococci (Wolbach strain) have been subsisting in the mouse kidney for 54 hours before penicillin was started, the drug was just as effective in terms of population reduction as when started at the beginning of the infection. (It should be recalled that the kidney of the mouse is the organ in which the most abundant growth of staphylococci occurs, and at 54 hours the populations are at their highest and microscopic abscesses are apparent although no grossly purulent lesions are yet present.)

The difference in penicillin effectiveness in the Eagle experiments and those just cited is more credibly attributable to differences between streptococci and staphylococci in the two sets of circumstances, rather than to environmental effects on the drug.

The second set of observations on this point was made by Dr. Berntsen who produced a sterile purulent-necrotic lesion in the gastrocnemius muscle of the mouse. In control experiments it was demonstrated that this sterile lesion would support the growth of beta hemolytic streptococci to the same extent as occurred in unaltered muscle. When the large populations, i.e., 10^9 of streptococci, most of which were less than four hours old, were introduced into this purulent lesion and the animals were treated with penicillin, there was a rapid fall in microbial census. Indeed, the population-reducing effect of the penicillin in these purulent lesions was exactly the same as it was *in vitro*. When the large "older" populations were introduced, the results were quite different, and the administration of penicillin had only a slow and imperfect effect on the reduction of the microbial census. Thus once again when the state of the parasite was one known to be associated with a high degree of drug susceptibility, the presence of large purulent lesions had no influence on the full penicillin action.

In this same experimental system, populations of streptococci were studied which had been grown *in vitro* for 36 hours. The populations had stabilized under these circumstances and hence were under whatever inhibitory factors make for such stabilization *in vitro*. When these populations were placed in the purulent-necrotic lesion, they were relatively indifferent to the action of the penicillin. Thus, the change from the *in vitro* environment in

which stabilization had occurred to the purulent environment *in vivo* did *not* have the effect of shaking or aeration or cortisone. Instead, the stabilized populations simply maintained their state of relative drug indifference in the necrotic-purulent environment.

Another example of this sort has to do with staphylococci, penicillin, and leukocytes. It will be recalled that Dr. Tompsett, using this system, was able to show that about 10 per cent of the population of staphylococci survived penicillin when they were inside leukocytes, whereas only .0001 per cent survived when they were outside the leukocytes. This could represent either failure of delivery of drug to *all* of the intracellular staphylococci or the possibility that the environment provided by the leukocyte induced or favored the assumption of a state of drug indifference by some of the staphylococci.

In subsequent experiments Dr. Tompsett[35] used disrupted leukocytes in the same numbers as the intact leukocytes used previously. He observed that the same 10 per cent penicillin survival which occurred when the staphylococci were situated in the leukocyte likewise occurred when *disrupted* leukocytes were used instead of intact cells. As he pointed out, the demonstration of the same phenomenon occurring in the two sets of circumstances does not establish the fact that the mechanism is necessarily the same in both instances. Nevertheless, the results are suggestive in themselves and have the additional implication that purulent material per se may represent one of the environments in which microbes assume a state of relative drug indifference.

The last-named point was investigated by Dr. W. Barry Wood[41] in some experiments published last year. He observed that pneumococci inoculated into purulent material did not show a rapid increase in numbers, but instead persisted at the initial census or gradually fell in numbers with the passage of time. Moreover, Canetti[4] and others have shown that the caseous lesion of tuberculosis, when its bronchial communication is not patent, i.e. a "closed" lesion, has a considerable self-sterilizing capacity.

It can easily be shown that the presence of one antimicrobial drug in the environment of a microbial population can influence the susceptibility of the population to another drug. This was originally demonstrated for sulfonamide and penicillin by Hobby and her associates and has been the subject of a series of detailed investigations with a large number of drugs by Dr. Ernest Jawetz.[19] Moreover, in studies with antituberculous drugs in our laboratory,[21] it has been possible to show that the population-reducing effects of isoniazid during the early weeks of its administration could be largely neutralized by the concurrent administration of nicotinamide or its derivative pyrazinamide. Nicotinamide is a weak antituberculous drug,

whereas pyrazinamide is a relatively strong one. It was further shown that tubercle bacilli of human origin exposed *in vivo* to isoniazid (or other antituberculous drugs) had greatly increased susceptibility to the pyrazinamide. The increased susceptibility to pyrazinamide could also be shown when the isoniazid exposure preceded rather than accompanied the exposure to pyrazinamide. Dr. Koch-Weser[13] and his associates have shown that sequential exposure of tubercle bacilli to another pair of antituberculous drugs may affect tubercle bacilli *in vitro* in a manner distinctly different from what happens when the bacilli are exposed to both drugs at the same time.

These examples are cited merely to show that a commonly encountered component of the inflammatory environment, an antimicrobial drug, can exert widely divergent influences on the susceptibility of a microbial population to other antimicrobial drugs. None of these drug-pairings have so far proved to be eradicative, and when the influence of the drug-altered environment happens to be in the opposite direction, namely, toward a lessening of microbial susceptibility, the phenomenon of microbial persistence is thereby favored. Thus the situation appears to be no different with respect to environmental influences in general and environmental influences consisting largely of another antimicrobial drug.

Because the present discussion is primarily concerned with microbial persistence, attention has been focussed on environmental influences and microbial adaptations of a sort that would tend *to lessen* the susceptibility of a microbial susceptibility to certain drugs. In the case of tubercle bacilli, a population which has subsisted in the mouse for 21 days is more susceptible to pyrazinamide, and probably also to streptomycin, than a population freshly introduced into the animal. It will be recalled that the susceptibility of tubercle bacilli of human origin to either of these drugs *in vitro* can be drastically modified by appropriate manipulation of the environment. Moreover, a particular environmental change will modify the pyrazinamide-susceptibility of tubercle bacilli of human origin, whereas it will not affect those of bovine origin. Or a particular change will modify the streptomycin-susceptibility of tubercle bacilli, but not of *E. coli*. As in the case of environmental lessening of drug-susceptibility, the change seems to depend on the adaptive plasticity of the microbe and not on the presence of an environment more favorable for drug "activity."

To sum up this portion of the discussion: it is believed that microbial persistence is the result of the ability of microbial populations to assume a state in which they are relatively insusceptible to an antimicrobial drug. It is believed that the assumption of this state, which is called "drug-indifference," is induced or favored by the influence of the environment on the

microbe. Included in these "environmental influences" are antimicrobial drugs and intermicrobial relationships* as well as the influences of the host cellular and humoral defense reactions and the reactions of inflammation.

An environmental change of a particular sort may make the same parasite display widely different behavior to different drugs or may make different parasites display less than their maximal susceptibility to the same drug. For these reasons it is believed that it is the adaptive plasticity of the microbe which is the important factor in the influence of environment on drug effectiveness *and not a physical or chemical antagonism exerted directly by the environment on the drug.*

The environmental factors which can influence microbial populations toward lessened or enhanced susceptibility to drugs include the orthodox host-immune mechanisms, such as phagocytes and antibody, and the previous or concurrent presence of another drug. Probably of even greater importance are forces as yet undefined but nonetheless visible in the form of effects, among them those, neutralized by steroid hormones, which prevent the microbial populations from rising in certain organs to the peak attained in other organs of the same animal; the consequences of tissue alteration, i.e. inflammation and necrosis; and the consequences of microbial crowding or "the territoriality phenomenon" which can be demonstrated *in vitro* and can be reversed by environmental manipulation.

Although instances of drug-enhancement from environmental adaptation of the microbes occur, thus far they have not been observed to lead to total eradication of a microbial population. Consequently, the net over-all effect of the various environmental influences on drug effectiveness is in the direction of providing situations which favor microbial persistence.

The question arises as to the relation of microbial persistence to the production of tissue changes and the calling forth of immune mechanisms. There is some reason to believe that stabilization of a microbial population per se, as may happen, for example, at a high census in an untreated infection, does not necessarily coincide with a cessation of progressive changes in the tissues. With respect to "persisters," however, we are considering a population which is present at a very low census. The possibility cannot be excluded that such small numbers of apparently "inactivated" microbes do not give rise to tissue reactions. It can be said, however, that if such changes occur, they are subtle in nature, for the characteristic tissue

* With respect to the purely intermicrobial aspects of this situation, the concept is, of course, a broadening of the concept of the viral "interference phenomenon" and the application to microbes of the biological concept of "territoriality" as it applies to animals of the same and different species (see *The prevalence of people* by Marston Bates[9]).

changes produced by tubercle bacilli or staphylococci are not seen in the animals harboring "drug-persisters." With respect to persisters and the evocation of immune mechanisms, it can be said, as will be discussed below, that in certain circumstances at least, microbial persistence can occur without evoking the host-immune response characteristic of that particular infection.

Reflection on the question of the relation of persisters to tissue changes or immunity leads naturally to speculation concerning the form in which microbial persisters exist during their periods of hibernation. Until quite recently there was no convincing demonstration of a correlation between a particular microbial morphology and a physiological state of drug-indifference. One had to be content with analogies drawn from the phenomenon of bacterial sporulation[24] or advance the dead-end argument that nonvisible, drug-indifferent forms existed for microbial species not known to have multiform life cycles. Within the past few years, however, there have been a number of studies dealing with the L-forms or protoplasts of certain microbes that appear to have a definite bearing on the action of penicillin.

Before considering these recent penicillin studies, it should be noted that Schnitzner and his associates[25] observed the occurrence of small colony variants, the so-called gonidial or G-forms, in cultures of staphylococci exposed to penicillin. Wise and Spink have made an extensive study of this subject.[26] In their studies with staphylococci the colonies were so minute as to be barely visible macroscopically and the microbes appeared to be unaffected, or at least they survived, concentrations of penicillin which were usually inhibitory. On subsequent cultivation in the absence of penicillin, the minute colonial forms gave rise to staphylococci of orthodox colonial morphology and in this state the microbes were susceptible to penicillin. Moreover, they found evidence of the presence (but not the reversion) of these G-forms *in vivo*. A somewhat similar type of demonstration was made by C. H. Lack[14] with tubercle bacilli. Using phase contrast microscopy and a hot box, he observed that tubercle bacilli subjected to starvation or antimicrobial drugs (streptomycin, PAS, penicillin, isoniazid) became progressively smaller until the cells were coccal rather than bacillary. When nutrition was restored, some of the coccal forms grew back to bacillary forms. Although Lack believed that these coccal forms probably represented a stage associated with drug survival, he did not consider the point as having been established.

The results of these various studies suggested the existence of morphologic counterparts to certain physiologic states involved in drug susceptibility, yet none were presented as constituting clean cut evidence on that point. Within the past year, however, several studies have been reported

dealing with the L-forms or protoplasts of bacteria which seem to have a definite bearing on the subject.

When the cell wall of a bacterial cell is lysed under appropriate conditions, the bacterial cytoplasm and its limiting membrane may continue to exist as the so-called protoplast (Weibull[28]). This process is regarded by several groups of investigators (Hahn and Ciak,[10] Park and Strominger,[27] Lederberg,[16] Pease[28]) as being identical with what happens in the first stage of the formation of the L-form from the normal vegetative form of certain bacteria. The protoplast is quite fragile and is especially responsive to the osmolarity of the environment. Nevertheless, protoplasts can survive *in vitro* with appropriate manipulations of the environment and if protoplasts and L-forms are identical microbial forms, protoplasts obviously survive *in vivo,* where the osmotic homeostatis might be expected to be more protective. The environmentally induced conversion of L-forms to a vegetative corynebacterium in Hela cells and a similar type of change with *H. pertussis* in the mouse lung by Wittler and her associates have been previously mentioned.

The importance of these studies of protoplasts with respect to microbial persistence lies in the fact that several investigators have shown (Lederberg,[16] Liebermeister and Kellenberger,[19] Hahn and Ciak,[10] Pease[28]) with two microbial species (*E. coli; Proteus vulgaris*) that protoplasts can be regularly *induced* by appropriate exposure to penicillin. Moreover, the protoplasts so induced *are not destroyed by penicillin and when the penicillin is removed from the environment, the protoplasts revert to the vegetative (and penicillin-susceptible)* form of the microbe. In addition to these two microbial species in which penicillin-induced protoplast formation has been shown, Park and Strominger have presented highly suggestive evidence with staphylococci that it is the synthesis of the bacterial cell wall which is interfered with by penicillin.

Thus there now exist experimentally demonstrated *biological precedents* for every step of the argument that microbes possess an adaptive plasticity in relation to their environment which permits them to persist in the animal body despite exposure to the appropriate antimicrobial drug.

It is important to realize that what has been demonstrated has to do with *precedents* or *examples* and not with the actual mechanics of the phenomenon of microbial persistence per se. Protoplast induction has been demonstrated only for penicillin (of the commonly used antimicrobial drugs) and only for two microbial species, neither of which produces what is conventionally regarded as a penicillin-susceptible infection in man. At the moment, therefore, protoplast formation might well be regarded more as an explanation of why penicillin fails to be effective in certain Gram-negative

bacillary infections rather than why it fails to eradicate the Gram-positive microbes from animal tissues. Nevertheless, these recent studies clearly show the existence of a drug-induced microbial morphology which is correlated with the physiological state of "drug-indifference" and both the morphological expression and the physiological state are reversible by appropriate manipulation of the environment of the microbes.

In the "persisting" states which have just been conjectured, the microbes are not only physiologically unreceptive to the drug, but have been morphologically altered as well. There is some reason to suspect that this concept represents too crude an oversimplification of what actually happens, or at least that it represents only one type of a phenomenon which may occur in several forms. The principal reason for this suspicion is the fact that, in certain circumstances, microbes which are genetically resistant to a particular drug may nevertheless "persist" in animals treated with that drug without showing any tendency to increase in numbers. If microbial persistence were entirely dependent on a morphological change in the microbe, for example, protoplast formation with the loss of the cell wall, it is difficult to visualize how this could occur if the individual microbial cell were genetically resistant to the drug in question.

When a strain of tubercle bacilli or staphylococci that will grow freely in the presence of a particular drug *in vitro* is injected into a mouse and the corresponding drug is administered, the microbial population in the mouse will increase in essentially the same way as in untreated animals. The microbes cultured from the tissues at any time during the course of therapy, will show the same drug-resistance *in vitro* (considering the strain as a whole) as was the case with the pre-inoculation strain. In contrast, as we all know, when the infecting strain is drug-susceptible and the drug is administered, the initially high census of culturable microbial cells falls to a greater or lesser degree and persists apparently unchanged thereafter. The persisting cells (or rather their descendants) are almost invariably drug-susceptible when subsequently tested *in vitro;* when drug-resistant variants emerge, the microbial population usually shows a rapid upsurge despite the chemotherapy.

The point for consideration, however, is the fact that this upsurge of a small drug-resistant population does not invariably occur. Sometimes, with both tubercle bacilli and staphylococci, these drug-resistant microbes just persist, showing no tendency to increase in census. Whether the continued administration of the drug is a factor in this situation, despite the drug-resistance of the persisting strain, cannot be stated from our own studies because thus far we have not made observations on such populations after the cessation of therapy.

At the semantic level, if not otherwise, it is difficult to visualize exactly how genetically drug-resistant cells would have the opportunity to assume physiological states of "drug-indifference." This suggests, therefore, that there are aspects and implications of the physiological states of drug-indifference which have nothing to do with the drugs. This is to say that the microbe is capable of assuming states in which it is sufficiently in balance with its host environment so that it neither proliferates nor is destroyed and can persist in such states for long periods. Obviously, this is but another way of saying something we all know, namely, that microbial infections (including the drug-resistant cells therein) can become dormant or truly latent in an animal host by natural processes and not only as a consequence of antimicrobial therapy. In short, it is probable that the mechanisms of microbial persistence despite drug therapy are very much like the mechanisms which permit infections to become dormant or latent in the absence of therapy.

It is not the purpose of the present discussion to consider the host mechanisms possibly operative in maintaining an infection in the dormant or truly latent state.* Obviously, both the known and probably certain as yet unknown host reactions play a most important role in this process. The point for emphasis in the present discussion is that perhaps we have tended to be too one-sided in our appraisal of this situation and, in quite properly focusing attention on the host, we have neglected the possible adaptive plasticity of the parasite. I am thus suggesting that there has been a tendency to regard the parasite in a dormant or latent infection as being very much like the same parasite when it is actively producing disease, and hence to regard the difference between the latent and active stages of a particular infection as depending almost entirely on the momentary status of the bodily defenses. It is not so much that this viewpoint might be untrue as that it is almost certainly only one part of a larger picture.

To be sure, in many instances, a change in the tissue environment provided by the host for the microbe will result in an evocation of an infection previously dormant. The observations of the Danish medical students imprisoned in the German concentration camps during the latter days of World War II represent a case in point.[15] The diseases that appeared were all produced by microbial species almost certainly a part of the personal flora of those afflicted. The extremes of the deprivation of the host were

* In this discussion, the word *dormant* is used for infections persisting at a low but detectable level. The word *latent* is reserved for situations in which the presence of the infection cannot be demonstrated by any of the available methods and the fact that it *is* present can only be detected in retrospect by the appearance of relapse.

such that the tissue environment of the microbes was almost certainly altered substantially. Hence, there is no particular reason to assume that any more marked changes occurred in the microbe than regularly occur in the change from a "resting cell" to one in the stage of logarithmic growth.

But there are instances, in which the situation is less clear, for example, Brill-Zinsser disease or latent syphilis. Within a relatively short period (probably two years) after a syphilitic infection is first established, it becomes latent in the majority of those infected. By this is meant that the presence of the infection can be detected by serological tests, but there is no way of demonstrating *where* the infection is persisting in the living patient nor indeed on post-mortem examination of those dead from other causes. Once this state of latency is established, it is highly unlikely that spirochetemia ever occurs thereafter. It should be noted, moreover, that the major late complications of syphilis, cardiovascular or neurosyphilis, almost certainly do not represent evocations of latent syphilis with invasion of new areas, but rather the emergence to clinical recognition of disease processes which have been slowly progressive ever since the initial infection. Indeed, the only evidence that the syphilitic infection is still present in the stage of latency are the facts that the tissues react to re-inoculation in a markedly altered fashion and in a small number of those infected cutaneous or skeletal gummata eventually develop.

It is of considerable interest, in this connection, that despite the many decades of observation of persons infected with syphilis and subjected to severe privations, such as occurred in a concentration camp, no one has ever reported anything remotely resembling the evocation of latent syphilis. When the latent infection is evoked, as it is in the formation of exterior gummas, the lesions almost invariably occur at a site of continued trauma. Since the stage of spirochetemia in such cases was presumably over two or three decades previously, it is reasonable to assume that *T. pallidum* must have been implanted many years before in the area of skin or bone which gives rise to the gumma. Because of the peculiar predeliction of such lesions for the sites of continued trauma, however, one must either attribute an unusual prescience to *T. pallidum*, or assume that the initial invasion of these tissues by the spirochete, and its continued presence thereafter, is something which occurs on quite a widespread basis. If this is so, it is all the more remarkable that no amount of deprivation of the host as a whole appears to do what is apparently done by the continued trauma to a part.

These observations suggest to me that *T. pallidum* is widely distributed throughout the tissues initially and exists thereafter in a form quite different from the one we have learned to recognize. The fact that *T. pallidum* can be demonstrated to be present in the aortic and neural lesions, but not

usually in gummas, is in keeping with this view. With full recognition of the highly speculative nature of these "reflections on syphilis," it does seem as if evocation from the latent state by a particular microbial species might require some rather considerable adaptive changes on the part of the parasite and not just merely a breakdown of some control mechanisms of the host. Indeed, it is not too inconceivable to suggest that the difference between a dormant infection such as a tuberculous pulmonary lesion and a truly latent one such as syphilis, might rest right at this very point, namely, that for evocation the dormant infection requires only alterations in the host, whereas the latent infection requires changes in the parasite itself. It is possibly relevant in this connection that a truly latent bacterial infection in rats and mice which can be evoked by cortisone[18] is produced by one species of the genus Corynebacterium, another member of which has been recently shown to progress through a complicated life cycle in mammalian tissue.[40]

So much for this highly speculative consideration of microbial states during latency. A question far more germane to the present discussion is whether the continued administration of an antimicrobial drug exerts any influence at all in the direction of maintaining an infection in the dormant or latent state. In other words, are microbes that are not producing disease, actually drug-susceptible? As most of the information bearing on this point also has to do with the question of whether microbial persistence just "happens" or is actively induced by drugs, these two questions will be considered together.

There is some reason to believe that when a microbial population first infects a new host, some members of the population are in (or quickly assume) a state of "drug-indifference" and can be kept in that state for so long as appropriate antimicrobial therapy is administered. The evidence on this point consists of a series of clinical observations; indeed the earlier observations are the ones which originally provoked our interest in the subject of microbial persistence. In essence, the observations are of two sorts: recognition of our customary inability to render humans predictably and uniformly free from the carrier state of otherwise drug-susceptible microbes, and recognition that microbial persistence usually occurs when infections are treated immediately after their inception.

The question of carrier states has been considered earlier in this discussion as was the observation in syphilis that if therapy were started very early, the results in all the large series of treated patients were never quite so good as when treatment had been started after clinical evidences of generalized infection had become apparent in the so-called secondary stage

of syphilis. In the pre-penicillin days, these differences in therapeutic results were never marked but they were consistent and appeared to be definite. Many an experienced syphilologist would tell one privately that *if he acquired syphilis* he would attempt to keep that fact a secret until the infection had progressed to the secondary phase with a generalized cutaneous infection, before he would permit the start of arsphenamine therapy. In short, a drug which was effective when the infection had progressed to a certain point was somewhat less effective when started prior to that point.

A similar phenomenon has been observed in the chemotherapy of four other infectious diseases: malaria, scrub typhus, Q-fever, and tuberculosis. In the first three infections, there is evidence that antimicrobial therapy exerts its maximal influence only after the infection has evolved to a certain point—with an apparently static microbial persistence occurring if treatment is started earlier. With tuberculosis, the evolution to a more satisfactory stage from the standpoint of chemotherapy has not yet been shown but the apparently static stage of microbial persistence following very early chemotherapy has been observed.

The evidence from malaria consists of the observation of Coggeshall[6] and his associates that the post-treatment relapse rate in tertian malaria was appreciably less when chemotherapy was started after the patient had had several individual paroxysms than when the same chemotherapy in the same time-dose relationships was started after the initial paroxysm of the disease. The observations in tuberculosis were made by Lelong and his associates[17] in Paris. They had the opportunity of starting isoniazid therapy in four infants born of mothers with pulmonary tuberculosis in an infectious stage. The antimicrobial therapy was started shortly after birth and the infants were immediately removed from contact with their mothers or other tuberculous persons and maintained in a protected environment. At the start of isoniazid treatment, the infants showed no evidence of tuberculous infection and their cutaneous reactions to tuberculin were negative. The isoniazid was administered daily for a 3- to 4-month period during which the infants developed normally and continued to remain nonreactors to tuberculin. Within four to six weeks of cessation of the isoniazid, however, and without any known renewed exposure to tuberculosis, all four infants developed positive cutaneous reactions to tuberculosis. It appears, therefore, that the isoniazid was able to hold this very early tuberculous infection suppressed at a level so low that cutaneous sensitivity to tuberculin failed to develop. Yet even in what might be considered a most advantageous situation from the standpoint of drug effectiveness, the isoniazid was unable to eliminate the infection.

The observations in the rickettsial infections, scrub typhus and Q-fever are the most clean-cut as they were made in the form of actual experiments with the use of human volunteers.

In Malaya, Drs. Smadel, Woodward, and their associates[33] showed that the initiation of chloramphenicol within a matter of hours after heavy exposure of volunteers to the rickettsia of scrub typhus prevented any clinical manifestations of illness so long as the chloramphenicol administrations were continued. Once the drug therapy was discontinued, however, even after so long a treatment period as 40 days, the infection would emerge to become the complete clinical illness of scrub typhus. It should be recalled that only 36 to 48 hours of chloramphenicol therapy are necessary for the successful treatment of the clinically apparent scrub typhus. Thus, in the presence of a fully established infection, 36 to 48 hours of drug therapy would produce an effect which was not producible *when 40 days of therapy with the same drug were given at an earlier stage of infection.*

This general phenomenon has been further defined in studies with Q-fever which were reported last May by Drs. Tigertt and Benenson[34] before the Association of American Physicians. These investigators were able to show that 5 days of tetracycline therapy represented perfectly satisfactory therapy for the treatment of established Q-fever. They also showed that the incubation period of experimentally induced Q-fever in man or in the guinea pig could be shortened or lengthened by appropriate manipulation of the dosage of infective particles. Thus the incubation period could be quite precisely predicted. In this standardized experimental situation, they found that 5 days of tetracycline therapy started immediately after an infection with a predicted incubation period of 12 or 14 days had no real effect on the subsequent course of events. To be sure, the incubation period might be extended for a few days beyond that predicted, but in all cases the subjects developed the complete clinical illness of Q-fever. The Tigertt-Benenson observations up to this point, therefore, were the same as the Smadel-Woodward observations on scrub typhus, and the old clinical inferences from the behavior of infectious syphilis. The additional information of considerable significance provided by the Tigertt-Benenson experiment, however, came from the fact that they found that if the 5 days of tetracycline were given late in the incubation period, immediately before the anticipated outbreak of the clinical illness, no clinical illness developed.

Thus there exist five examples of infectious diseases in humans, in which it has been possible to observe the effectiveness of antimicrobial therapy started very soon after the inception of infection. In all five examples (syphilis, malaria, scrub typhus, tuberculosis, Q-fever) the therapy is not

eradicative, but simply holds the situation "frozen," so to speak, for as long as its administration is continued. In addition, except possibly for tuberculosis, there is evidence from these various diseases that there exists a stage to which the host-parasite reaction must mature before the infection is fully drug-susceptible as measured by post-treatment relapse. The Tiggert observations with Q-fever show that this particular stage of maturity is not necessarily so old as the stage of evolution to the full clinical illness.

Aside from the possibility of eradication during therapy, the prevention of post-treatment relapse reflects the extent of the mobilization of certain host mechanisms that require both the passage of time and a sufficient "antigenic" stimulus to become fully operative, or the development of certain evolutionary changes in the parasite. When an increase in numbers of the infecting population is artificially prevented by administration of an appropriate drug, any evolutionary changes which might have occurred in the parasite and the production of a sufficient "antigenic" stimulus are both hindered. It is important to realize that in these circumstances the infecting population as a whole cannot be said to be *uninfluenced* by the drug. For, the evolution of the infection to overt disease apparently *can* be arrested for an indefinite period despite the absence of a sufficient host reaction to prevent post-treatment relapse. Thus the microbial population may be regarded as paradoxically being both "drug-influenced" and "drug-indifferent" at one and the same time.

Presumably what is happening is that a portion of the population is and remains drug-indifferent. When members of this drug-indifferent group assume a state of greater drug-susceptibility or are new-born into such a state, they are presumably immediately incapacitated by the antimicrobial drug present in the environment. The microbial population as a whole may, therefore, be said to be in a state of *"physiological imprisonment."* In this situation the individual microbes are not eradicated, neither can they evolve to the point of permitting a host-parasite reaction to develop which would be susceptible to drug treatment with appreciably less risk of post-treatment relapse.

In the clinical examples cited this situation apparently obtained from the moment of infection or soon thereafter. This suggests either that some of the infecting microbes were drug-indifferent at the time of infection or became so very shortly thereafter, and that microbial persistence may have its beginning in the very earliest stages of an infection. If the concept presented earlier should have validity, namely, that within a few hours of birth microbial cells may have matured beyond the point of penicillin-susceptibility, every penicillin-susceptible infection would presumably contain microbes already "drug-indifferent" at the moment of infection. By the same

token, such phenomena as the relative drug-indifference of microbes held intracellularly, or the induction of protoplast formation by penicillin exposure, suggest that if microbes are capable of assuming a state of drug-indifference as an adaptation to their environment, they could do so within a few moments of infection especially perhaps in the presence of chemoprophylaxis.

In actuality, there is no evidence at all on this question of whether certain cells in a new infection are drug-indifferent right from the beginning or whether they promptly become so as a response to the new environment including the antimicrobial drugs therein. As may be inferred from the foregoing discussion, I *believe* that both phenomena obtain. The point is not necessarily an important one, however, because in either case the net result would be the same and is something which may be of considerable importance in considerations of chemoprophylaxis.

The actual physical transfer of microbes to a new host is presumably not always followed by their successful colonization there. In such circumstances, the additional inhospitable factor of an antimicrobial drug in the new environment might well make the difference between the success or the failure of the implantation. The point for emphasis, however, is the strong possibility indicated by the considerations presented above, that *even if the appropriate antimicrobial drug is present in the tissue environment at the moment of implantation, it does not necessarily follow that no microbes will survive there as persisters.* Indeed, from experience in the penicillin prophylaxis of gonorrhea, when the subject was simultaneously exposed to syphilis, there is some evidence that *T. pallidum* implanted in the tissues of a host where extracellular fluid contains penicillin can nevertheless survive and eventually give rise to syphilitic infection. The acquisition of gonorrhea is, to be sure, prevented by the penicillin. But, as mentioned previously, Neisseria represent one of the two microbial groups that apparently lack the capacity to exhibit microbial persistence.

To sum up this aspect of the discussion, one must beg the question as to whether microbial persisters are actively drug-induced or arise only as an evolutionary adaptation to their environment. What can be reasonably inferred, however, from the five clinical examples cited and the experience with "healthy carriers" is: that microbial persistence can occur right from the early stages of an infection before environmental changes as a consequence of microbial-host interaction would be too advanced, and that in the examples cited it is clear that the over-all effectiveness of an antimicrobial drug is at its maximum when the infection is actually producing disease.

What are the prospects that antimicrobial drugs will be developed which will abolish microbial persistence—drugs which will be predictably eradicative instead of just being suppressive as is the case with our present drugs? Thus far, the prospects do not look hopeful. In the first place, the two microbial groups (Neisseria and B. dysenteriae) which appear to lack the capacity to persist are apparently eradicated from the tissues by drugs such as sulfanilamide (Neisseria) or the tetracyclines (B. dysenteriae) neither among the most powerful of our antimicrobial drugs. In other words, the eradicative action of these drugs in these infections seems to represent more the essential fragility of the parasites rather than any qualitatively different types of drug action. Tubercle bacilli were studied from this standpoint using the nicotinamide derivative, pyrazinamide. When pyrazinamide and any one of several antituberculous drugs are administered in appropriate time-dose relationships to tuberculous mice, the tubercle bacilli completely vanish from the tissues of the mice and the infection is rendered truly latent.[21] By this is meant that it is not possible to detect the presence of the tubercle bacilli by microscopy, by elaborate culture techniques, or by subinoculation of guinea pigs. To this extent, therefore, the therapy is eradicative. It is easily demonstrated that the infection has not really been eradicated, however, by observing the animals for a 90-day treatment-free interval. When this is done, tubercle bacilli in small numbers can be cultured from the tissues of approximately 40 per cent of the mice. Moreover, these microbes are true persisters for as a general rule, they are susceptible to the drugs involved when subsequently tested *in vitro*.

In short, microbial persistence may occur at different levels of detectability, yet the phenomenon still endures. Moreover, thus far in the microbial species studied experimentally the phenomenon has remained invulnerable despite concerted attacks with all of the available antimicrobial drugs used singly or in various multiple-drug regimens.

SUMMARY

From certain inferences derived from clinical phenomena and experimental studies in animals, the phenomenon of microbial persistence can be demonstrated to occur broadly throughout the microbial world. The phenomenon occurs in the absence of antimicrobial therapy, but it is its occurrence despite antimicrobial therapy that makes understanding of the phenomenon of such importance in considering treatment failures, post-treatment relapse, and the chemoprophylaxis of either infection or its subsequent individual manifestations as disease.

As to how the phenomenon of microbial persistence occurs, there is little if any truly direct evidence. From indirect evidence interpreted specu-

latively, it appears that microbial persistence is environmentally induced and is one reflection of a high degree of adaptive plasticity of individual microbes. It is believed that the range of such types of microbial adaptation *in vivo* is substantial and that it is this capacity of individual microbes to express individuality which determines in large measure the success or the failure of antimicrobial therapy. An important role of the host defenses in this process would be to participate in providing "proper" environments for the adaptive capabilities of the microbe. As the environments represent the interaction of the host and the parasite, neither can be dismissed from consideration. It is suggested, however, that perhaps we have been taking too one-sided a viewpoint in our preoccupation with host mechanisms and should focus equal attention on this probable wide range of reversible changes on the part of the microbe.

As host reactions are *ad hoc* in nature and not necessarily beneficial to the host as a total organism, the particular environment provided by a host reaction may or may not lead to a microbial adaptation which favors maximal drug-susceptibility. Thus the environment of an actively progressing infection may be ideal for the maximal drug-susceptibility of the microbe, whereas necrotic areas or the interior of phagocytes in the same host may favor microbial adaptation to assume a state of "drug-indifference." Viewed in this way, the influence of the inflammatory environment is probably not exerted by impeding, neutralizing, or enhancing the activity of a drug, but rather by providing an environment favorable or unfavorable for the assumption of certain states necessary for drug-susceptibility of the parasite.

In other words, it is suggested that the nature of the lesion does not directly influence antimicrobial drug activity. Instead, it is the adaptive response of the microbe to a particular lesion or a particular host environment which determines the direction an individual drug-microbe collision will take. And, it is one manifestation of this adaptive plasticity of the microbe—the ability to "play dead" so to speak—that determines microbial persistence and thus provides a means for the survival of microbial species despite the development of powerful antimicrobial therapies.

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FUNGUS INFECTIONS OF THE URINARY TRACT†

Although bacterial infections of the urinary tract have received considerable attention in recent years, diseases of this system due to fungi have only been sporadically mentioned in the literature. The majority of cases reported have been instances of renal involvement secondary to dissemination of the fungi by the circulation. Only a few instances of primary diseases have been noted. With the increase in number of fungal infections observed in recent years,[22] it would be expected that a similar rise might occur in the incidence of primary urinary tract infections due to fungi. Since the clinical appearance of fungal and bacterial infections of the kidney and bladder are often indistinguishable, the diagnosis must be made from information obtained by urine culture. Fungi frequently appear in the urine in small numbers and are usually discarded as contaminants by laboratory personnel. However, with the use of quantitative methods of culture, it is possible to distinguish a significant bacteriuria (or "funguria").[21] This report describes observations made on 15 patients whose urines contained large numbers of fungi.

MATERIALS AND METHODS

Urine specimens were obtained in male and female patients by "clean voided" technique. Previous observations have demonstrated that this is a satisfactory method of collection when quantitative bacteriological procedures are employed.[28, 30] Urine was diluted serially in nutrient broth and 1 ml. of a 1:100 dilution incorporated into an agar pour plate. (Earlier in the work an additional plate was made with an aliquot of a 1:10,000 dilution.) Additional samples of undiluted urine were inoculated into blood and desoxycholate agar plates and thioglycollate broth. Cultures were examined after incubation for 48 hours at 37° C. In this manner an estimate of the type and quantity of organisms was obtained and further identification performed by appropriate methods.

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During the study of approximately 1,500 urines, fungi were occasionally found in small numbers (less than 1,000 organisms per ml. of urine) and these were considered to be contaminants. In 15 instances, however, larger numbers were encountered, and these were regarded as representing true fungus infections of the urinary tract.

Identification of fungi. Although various fungi have been isolated from urine, only yeasts were obtained in this study. The yeasts fall into three groups: *Endomycetaceae,* those producing ascospores; *Sporobolomycetaceae,* those producing ballistospores on sterigmata, and the *Cryptococcaceae,* those which do not produce ascospores or ballistospores. The yeasts isolated during this study were found to be *Cryptococcaceae,* the family which includes the general *Candida, Rhodotorula, Cryptococcus,* and *Torulopsis.**

The yeasts were inoculated to malt-yeast extract medium used for the maintenance of cultures. Before initiating studies to identify species of *Torulopsis* and *Cryptococcus* the organisms were inoculated to Zein medium to determine whether or not they were species of *Candida.* If such was the case, they were identified according to the method of Martin *et al.*[39] When an organism failed to fall into the *Candida* group, it was then identified according to the methods of Wickerham[47] and Lodder and Kreger-Van Rij.[25] The yeast was subcultured to ascospore medium to rule out the presence of a member of the *Endomycetaceae* family. It was then inoculated to Christensen's urea medium[39] which is an excellent screening medium for the cryptococci. Study of the morphology of the organism was done according to the method of Wickerham; following this fermentation and carbon assimilation studies were done. The ability of the fungus to utilize KNO_3 was investigated as well as the production of starch. With the information obtained from these studies, the yeast was then classified according to Lodder's taxonomic study.

OBSERVATIONS

In the 15 patients who had large numbers of yeasts in the urine, the counts ranged between 8,000 and 37,000,000 viable units per ml. of urine. Table 1 summarizes these cases. Thirteen of the patients were female, 2 were male. In an attempt to evaluate predisposing factors to this type of infection, a review of the patients' records revealed that 9 (7 female, 2 male) had diabetes mellitus, 3 had surgical procedures performed during the year prior to discovery of yeasts in their urine, and 12 had received antibiotics during this period. This latter group is interesting in that yeasts appeared in the urine in 9 of these 12 people during the course of treatment of a bacterial urinary tract infection.

The organisms isolated were of 2 genera, *Torulopsis* and *Candida.* As shown in Table 2, these yeasts were equally distributed in the patients, with no apparent predilection for diabetics or nondiabetics.

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* We are indebted to Dr. L. J. Wickerham, who verified the identity of several strains of yeasts isolated in this study.

TABLE 1. CLINICAL DATA OF PATIENTS WITH FUNGI IN URINE

Pt. No.	Age	Sex	Urine culture: No. of organisms per ml.	Urine culture: Type of yeast	Diabetes mellitus	Surgery	Antibiotics	Diagnoses and comment
1	29	female	8,000	*Torulopsis*, type unknown	No	10 mos. previous	penicillin	Rheumatic heart disease, mitral valvulotomy 10 months previously. No history of urinary tract infection. No intravenous pyelogram.
2	19	female	>100,000	*Candida krusei*	No	1 mo. previous	penicillin streptomycin chloramphenicol tetracycline chlortetracycline Neomycin®	Paralytic poliomyelitis. Left and right renal calculi. Left renal lithotomy 1 month previously. Persistent urinary tract infection for 1 year.
3	26	female	>100,000	Not identified	Yes (3½ yr)	No	Gantrisin® streptomycin chloramphenicol tetracycline	Renal cortical necrosis (artificial kidney 6 months previously). Developed pyelonephritis (coliform) while in hospital.
4	24	male	10,000	Not identified	Yes (16 yr)	No	penicillin streptomycin	Pyelonephritis (enterococcus). Intravenous pyelogram revealed double kidney (rt.) with urectasia and early hydronephrotic changes.
5	65	female	>100,000	*Torulopsis glabrata*	Yes (3 yr)	No	streptomycin tetracycline	Bronchopneumonia. Pyelonephritis (coliform). No history of previous urinary tract infection. No intravenous pyelogram.
6	65	female	>100,000	*Candida stellatoidia*	Yes (5 yr)	1 mo. previous	penicillin streptomycin tetracycline	Gangrene, left foot (amputation). Acute exacerbation of chronic pyelonephritis (coliform).

7	66	female	>100,000	*Candida stellatoidia*	Yes (15 yr)	No	streptomycin chloramphenicol tetracycline chlortetracycline erythromycin	Arteriosclerotic heart disease with congestive heart failure. Acute exacerbation of chronic pyelonephritis (coliform). Intravenous pyelogram revealed ureteritis cystica, right.
8	66	female	37,000,000	*Torulopsis glabrata*	Yes (18 yr)	No	penicillin streptomycin tetracycline	Gangrene, right hallux (amputation). Nephrectomy, right (1938) for chronic pyelonephritis.
9	62	female	>100,000	*Torulopsis glabrata*	Yes (noted on admission)	No	penicillin streptomycin tetracycline	(Autopsy) Chronic pyelonephritis; organized aortic bacterial endocarditis; infected infarcts of spleen; organized cerebral infarcts; left lower lobe atelectasis.
10	70	female	>100,000	*Torulopsis glabrata*	No	No	penicillin streptomycin	Basilar artery thrombosis. Died 2 months after admission. No autopsy.
11	35	female	5,600,000	*Torulopsis glabrata*	Yes (16 yr)	No	No	Admission for evaluation of headaches. No previous history of urinary tract infection.
12	14	female	>100,000	*Candida albicans*	No	No	Gantrisin® chloramphenicol	Spina bifida, meningomyelocoele, paraplegia, neurogenic bladder, chronic pyelonephritis with acute exacerbation (coliform).
13	29	female	>100,000	*Torulopsis glabrata*	No	No	No	Duodenal ulcer. No previous history of urinary tract infection.
14	23	female	>100,000	*Candida albicans*	No	No	streptomycin chloramphenicol tetracycline	Acute pyelonephritis (coliform) with bacteriemia. Cervical adenitis of unknown etiology. No previous history of urinary tract infection.
15	37	male	>100,000	*Candida tropicalis*	Yes (15 yr)	No	No	Kimmelstiel-Wilson disease.

CASE REPORTS

1. L. M. (case number 8, Table 1), a 66-year-old white female underwent a right nephrectomy in 1938 for chronic pyelonephritis. At that time diabetes mellitus was discovered; this had been controlled with diet and small amounts of insulin. In January 1956 amputation of the distal phalanx, right hallux was performed for osteomyelitis.

During this admission several urine specimens were obtained for bacteriological study. These cultures showed the following results:

1-7-56	no growth			
1-9-56	>4,000,000*	*Torulopsis*	*glabrata*	
2-22-56	>10,000,000	"	"	
2-23-56	>37,000,000	"	"	(bladder urine)
	>11,000,000	"	"	(left ureteral urine)
3-2-56	>100,000	"	"	(plus *B. proteus*)
3-15-56	>10,000	"	"	
10-30-56	no growth			
1-28-57	no growth			
4-1-57	no growth			

It should be emphasized that the patient received 1,200,000 units of penicillin and 1 gm. of streptomycin per day for 13 days prior to the discovery of yeasts in the urine. The presence of yeasts was noted before surgery was performed. Following cystoscopy (on 2-23-56), the patient developed frequency and burning on urination and *B. proteus* was isolated from the urine. This disappeared during treatment with streptomycin and chlortetracycline. The diminution in the number of yeast cells cultured in the urine during the *B. proteus* infection is interesting. Whether this was due to an unfavorable environment created by bacterial growth and subsequently accounted for complete disappearance of the yeast can only be speculated upon.

At cystoscopy it was noted that: "Bladder wall was trabeculated. The mucosa was a deep fiery red. In the trigone there was pseudomembrane formation." Biopsy of the bladder wall revealed marked submucosal lymphocytic infiltration, but was otherwise normal. Special stains did not reveal yeasts or bacteria within the tissue. Retrograde pyelogram demonstrated a spastic infundibulum to the upper pole of the left kidney which was thought to be possibly due to inflammation. Figure 1 shows the appearance of the urine obtained on February 22, 1956. Note that centrifugation of 10

* These figures represent the number of organisms per ml. of urine.

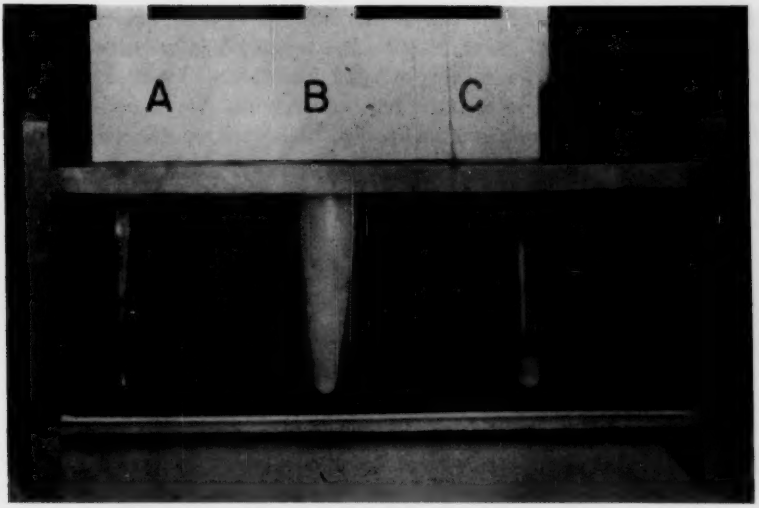

FIG. 1. Urine obtained from patient with *Torulopsis glabrata* infection (patient L.M., case report 1, case No. 8, Table 1). A. Urine from healthy control. B. Patient's urine, uncentrifuged. C. Patient's urine (10 ml.), centrifuged at 5,000 rpm for 8 minutes. Note that sediment volume, which consisted almost entirely of yeast cells, is approximately 1 ml.

ml. of urine at 5,000 rpm for 8 minutes yielded a sediment volume of approximately 1 ml. On microscopic study the urine was so laden with the yeast forms that it was impossible to decide about presence of pus cells.

2. R. R. (case number 11, Table 1), a 35-year-old white female, with diabetes mellitus of 16 years' duration and no history of previous genito-urinary tract infection, was admitted to the hospital for evaluation of frequent headaches in February, 1956. Examination, including lumbar puncture and culture of spinal fluid, failed to reveal the cause of her complaints. Initial urine culture demonstrated the presence of >5,600,000 colonies of *Torulopsis glabrata* per ml. of urine. This organism was consistently cultured from the urine in large numbers (≥100,000 per ml. urine) during the following 8 months. The patient has not been available for further study

TABLE 2. DISTRIBUTION OF YEASTS ISOLATED FROM URINE OF DIABETIC AND NON-DIABETIC PATIENTS

Yeast isolated	*Diabetic*	*Non-diabetic*
Torulopsis	4	3
Candida	3	3
Not identified	2	0

since that time. She had no symptoms referable to the genito-urinary tract throughout these observations. Intravenous pyelography revealed no abnormalities. Cystoscopy was not performed and there was no evidence of vaginal infection.

The persistent occurrence of large numbers of yeasts in the urine of these patients surely represents an abnormal state. Of the patients studied, 3 had fewer than 100,000 organisms per ml. of urine, 6 were examined only one time with no follow-up information available, and in 1 instance, although an initial urine culture revealed more than 100,000 viable units, a repeat culture 4 days later had only 3,700 colonies of yeast per ml. of urine. The remaining 5 cases had greater than 100,000 viable yeast cells per ml. of urine during 2 to 8 months of observation.

It is apparent from these data that it is often difficult to diagnose yeast infections of the urinary tract. Although the presence of large numbers of these organisms in the urine is abnormal, the significance of their transient appearance and the occurrence of asymptomatic infection is not clearly understood and will require further observation.

COMMENT

Historical. In 1931 Lundquist[27] described a case of primary fungus infection of the kidney ("mycosis renalis") and reviewed 17 cases previously published in the literature. Eleven of these were actinomycotic in nature, 3 were due to *Candida,* and there was 1 case each of *Sporotrichosis* and *"Cryptococcus renalis."* Renal involvement following hematogenous dissemination of *Candida albicans* frequently occurs[14, 19, 25, 46]; however, primary kidney infection without evidence of systemic spread has only rarely been observed.[1] Several patients with cystitis due to this organism have been noted.[23, 24, 41] Two instances of urinary tract infection by members of the *Penicillium* species have been reported. Chute[19] in 1911 described a case of cystitis due to *Penicillium glaucum,* and in 1945 Gilliam and Vest[20] examined a patient with infection due to *Penicillium citrium* in whom this organism was cultured from the right kidney and bladder at autopsy.

Although *Torulopsis glabrata* was isolated in several instances during the present study, little information is available concerning its pathogenicity. In 1937 Black and Fisher[6] described a case of bronchopneumonia believed to be due to *Torulopsis glabrata* in a 10-year-old boy. Plaut's patient[38] was a 31-year-old Chinese female who had granulomatous lesions of the cervix, endometrium, and fallopian tube associated with yeast-like organisms which were identified as *Torulopsis glabrata.* Wickerham[45] states that since March 1956 his laboratory has received 6 strains for identification, all isolated from human sources, 2 of which were associated with septicemia. The only reference made to the occurrence of urinary tract involvement with this organism is by Lodder and Kreger-Van Rij[25] who stated that one of the strains they studied was obtained from the urine of a patient with diabetes mellitus and cystitis. No further clinical description is given.

Pathogenesis. Torulopsis glabrata and *Candida albicans* have been isolated from the feces, skin, mouth and vagina in a varying proportion of apparently healthy individuals.[2, 5, 40, 45] That these organisms, and other fungi which are usually saprophytic, can invade tissue and establish infection locally has been asserted by Castellani,[11] who described urethromycoses in males and vulvovaginomycoses due to strains of *Candida* (Monilia), *Cryptococcus, Saccharomyces, Nocardia, Cladosporium, Aspergillus,* and *Penicillium.* From such foci these fungi were able to enter the bladder, their passage often being facilitated by urethral instrumentation.[14] The presence of fungi in small numbers in the urethra and bladder does not, in the majority of cases, result in infection. Factors which may alter host susceptibility in such a way as to permit development of a true infection include: pregnancy, diabetes mellitus, leukemia, and anti-leukemic drugs, general debility, anti-

bacterial therapy, corticotrophin or cortisone therapy, and changes in environmental temperature and humidity.[4, 7, 22] In addition, disorders of the urinary tract which cause stasis of urine flow tend to allow fungi to persist and multiply.

In the patients observed during the course of this investigation several of the factors mentioned may have played a role in the pathogenesis of mycotic urinary tract infection. Nine of 15 patients were diabetic, 12 had received antibiotic therapy within one year prior to appearance of fungi in urine and of these, 9 were being treated for bacterial kidney infection at the time of appearance of fungi in the urine. This latter group is interesting in light of Lepper's[24] observation of increased frequency of isolation of *Candida* from the urine of patients following the suppression of gram-negative bacteria with combinations of antibiotics which included tetracycline. A similar experience was encountered by Woods *et al.*,[50] who observed oral and bronchopulmonary moniliasis following the use of various antibiotics.

The influence of general physical debility, and possibly the post-operative state, on the occurrence of fungal infections is difficult to evaluate. This has been noted in infants and children.[23] The effects of concurrent disease, particularly leukemia, and the drugs used in these patients cannot be dissociated from the poor general state.[13, 31]

Renal involvement has been noted following dissemination of fungi via the circulation,[14, 15, 18, 19] and although no review of this material is intended at present, two cases are of interest. Wessler and Brown[48] and Schaberg *et al.*[35] each described a patient with disseminated candidiasis with survival, in whom the only persistent sequella appeared to be a chronic urinary tract infection with this organism.

Pathology. The small number of cases of primary renal fungus infection described in the medical literature permits only limited pathological observation. Also, the frequent association of a chronic bacterial infection prevents precise interpretation of histological findings. Nephrectomy was performed in two patients described by Albers,[1] both of whom had *Candida* infection superimposed on chronic bacillary pyelonephritis. In one, infected hydronephrosis with calculus formation was found, and in the other, pelvic leukoplakia. Although a species of *Candida* was cultured from the calyces in both instances, neither kidney had evidence of tissue invasion by the organism. In instances in which renal involvement occurred following hematogenous dissemination, the lesion appears to have a different character. Two of the cases presented by Schaberg *et al.*[35] had numerous abscesses throughout the kidney in which *Candida* was demonstrated culturally and histologically. Similar findings have been described by other authors.[14, 18] Necro-

tizing papillitis associated with *C. albicans* infection in a diabetic patient was described by Keye and Magee,[25] and we have observed a similar case. The patient described by Taylor and Rundle[48] had acute bilateral pyelonephritis with multiple 1-2 mm. abscesses throughout the renal parenchyma in which mycelia of *Candida* were seen. Although the organism was not cultured from the blood prior to death, the history of a preceding febrile illness associated with cough and sputum and the bilateral, miliary character of the renal lesions are evidences of hematogenous spread.

Cystoscopy in several patients with fungus infection of the urinary tract[12, 21, 41, 46] revealed changes of acute and chronic cystitis, occasionally with membrane formation, and, rarely, ulceration.[34] Biopsy of the bladder has shown chronic inflammatory changes in mucosa and submucosa with little tendency for the fungi to invade tissue. In our patient (case report No. 1) cystoscopy showed that the bladder was acutely and chronically inflamed, together with pseudomembrane formation. Histological examination revealed superficial inflammatory changes without evidence of fungi in the tissues.

Clinical manifestations. Lundquist[37] noted, "it seems that the process (of mycosis renales) clinically resembles suppurative pyelonephritis or frequent recurrent hematuria." The manifestations of primary fungus infections of the kidney appear indistinguishable from those due to the frequently accompanying bacterial infection. The patient with *Penicillium* infection of the urinary tract described by Gilliam and Vest[20] had episodes of recurrent hematuria, fever, and renal colic associated with ureteral passage of balls of mycelia of the organism. Taylor and Rundle[48] believed that the temporary diuresis in the course of otherwise progressive anuria noted in their patient may be indicative of intermittent ureteral obstruction by growths of fungi. A similar observation was made by Davis *et al.*[14] Intravenous and retrograde pyelograms in their patient revealed a nonfunctioning kidney with obstruction at the ureteropelvic junction which at nephrectomy appeared to be due to occlusion of the upper ureter by a "mass . . . of pus and large colonies of *Candida albicans.*"

Cystitis due to fungi has been described, *Candida* and *Penicillium* being the causative organisms.[12, 21, 34, 41] Symptoms of dysuria, frequency, urgency, and nocturia are reported. These infections have a tendency to chronicity with occasional acute exacerbations. Increased severity of symptoms associated with proliferation of organisms in the urine has been noted following the administration of sulfonamides[1] or mandelic acid[41]; whether this is related to suppression of asssociated bacteriuria is not clear. *In vitro* observations on this phenomenon by Paine[42] revealed that *E. coli* exerted a con-

trolling influence on cultures of *Candida,* and this was removed when antibiotics were added to the medium.

Due to the similarity of symptoms which may occur with urinary tract infections caused by fungi or bacteria, clinical differentiation may be difficult. In those cases in which fungi are isolated in pure culture from the urine, diagnosis becomes easier. However, with the occurrence of mixed or secondary infection, establishment of an etiological diagnosis may require continued observation and frequent quantitative urine cultures.

Experimental. Torulopsis glabrata. Relatively few observations have been made on the effects of inoculating strains of *Torulopsis glabrata* into experimental animals. Lodder and DeMinjer[28] reviewed some of this work and stated: *"Torulopsis glabrata* may possess some pathogenicity." In 1952 Fernandez[17] inoculated five strains of this organism into rabbits, guinea pigs, rats, and white mice. Most consistent results were obtained in the last; lesions occurring particularly in the heart, liver, and kidney. When inoculation was intravenous or into the peritoneal cavity, the organism tended to be found within macrophages and the histopathological appearance was similar to that of histoplasmosis. The typical renal lesion, observed between the 10th and 20th day after intravenous inoculation, consisted of small abscesses confined mainly to the cortex.

Candida albicans. Following the intravenous injection of *Candida albicans* into rabbits, kidney lesions almost invariably occur, and these progress to produce renal failure and death due to uremia within seven days. In early experiments Ashford[8] noted the occurrence of large, infarct-like lesions in the renal cortex, and Stovall and Pessin[48] demonstrated that these were not related to mechanical plugging of capillaries by the infected cells, but rather apppeared to be a manifestation of pathogenicity, i.e., the ability to grow and invade tissue. Employing strains of *Candida* of varying ability to produce disease in the rabbit, they demonstrated that a larger inoculum of an organism of feeble pathogenicity, with a mean cell diameter greater than that of a very virulent strain, did not result in such lesions in the kidney. Indeed, they were able to observe dissolution of these larger, less virulent, cells within renal capillaries when examined two and four hours after injection. In an excellent experimental study, Evans and Winner[16] noted another type of response of the kidney to this organism. In addition to abscesses, a hyaline thickening of the basement membrane ("'glomerular hyaline necrosis") was observed to begin 48 hours after inoculation. The authors felt that capillary embolism by the fungus was not responsible for this lesion.

Numerous investigators have attempted to define more clearly the pathogenesis of *Candida* infection by various manipulations of the host-parasite-drug relationship in experimental animals. Seligmann's[80] description of the enhancement of *Candida* infection in mice by chlortetracycline has been confirmed by others.[9] Since administration of the antibiotic by the intraperitoneal route was necessary to demonstrate this enhancement, it was suggested that a local irritative effect might be responsible. Winter and Foley[40] made further observations on this phenomenon and concluded that the widespread renal abscesses (cortex and medulla) seen following the intraperitoneal injection of *Candida* in mice are a result of hematogenous dissemination, while the extensive superficial lesions (almost exclusively confined to the cortex) which occurred in animals given one of the tetracycline drugs were due to direct invasion of the kidney along its peritoneal surfaces. In the antibiotic-treated animals, evidence of hematogenous spread occurred later and was not as extensive as in control group. Cortisone therapy resulted in findings identical to those observed in mice treated with antibiotics. Enhancement of infection and increased dissemination of organism has been noted following treatment of mice with formalinized suspensions of cells of *S. cerevisiae* and *C. albicans*.[86] In regard to this latter observation, it is of interest that Blank,[7] quoting unpublished observations of Roth, indicated that a toxin extracted from *C. albicans* by sonic vibration, when injected into mice combined with chlortetracycline, will result in uniform mortality. Injection of this toxin alone or antibiotic alone was without effect. The influence of environmental temperature on the course of candidiasis in mice has been studied.[87]

Interpretation of the experimental data presented in the literature is difficult. Some of the conflicting reports may be due to variations in such interrelated factors as severity of infection, species and sex of animal, amount of drug, hormone, or antibiotic employed, and environmental temperature. Further investigation of the pathogenesis of these infections is needed, and great care must be exercised to reduce the experimental variables.

Treatment. The treatment of fungus infections of the urinary tract has been largely unsatisfactory. Mandelic acid, acidification of urine, sulfonamides, potassium permanganate and silver nitrate have all been employed unsuccessfully. Some cases have required nephrectomy and in several instances, infection has recurred following surgery.[1, 14]

During the past several years, much attention has been given to antifungal antibiotics and information concerning these substances has been sum-

marized." The use of these substances appears to be a promising approach to the problem.

SUMMARY

During the course of examining approximately 1,500 urine specimens submitted to a hospital laboratory for bacteriological study, 15 instances were encountered in which culture revealed significant numbers of fungi per ml. of urine. The organisms isolated were species of *Torulopsis* and *Candida*. Pertinent clinical details regarding the 15 patients are given. Nine of them had diabetes mellitus, and 12 had received antibiotics during the preceding year; in 9 of the latter 12 cases the fungi had appeared during the course of treatment for bacterial infection of the urinary tract.

Previous publications on the subject of fungus infections of the urinary tract are reviewed. These take the form of primary involvement of the urinary tract, or may be part of a hematogenous dissemination. The former are usually confined to the bladder and urethra, and appear to be superficial infections with little tendency to tissue invasion. Renal involvement in the disseminated disease is characterized by the formation of miliary abscesses. Although various alterations in the host-parasite-drug relationship have been suggested as predisposing to fungus infections, the exact mechanism(s) involved are not well understood.

The occurrence and persistence of large numbers of fungi in the urine presumably represents true infection. The symptoms and clinical course of mycotic infections of the urinary tract are indistinguishable from those of bacterial involvement and the differential diagnosis depends on careful urine examination.

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QUANTITATIVE RELATIONSHIPS BETWEEN HYPOXEMIA AND DISORDER OF PULMONARY FUNCTION**

Hypoxemia is a common finding in many diseases of the lung. The degree of hypoxemia depends upon which aspect of pulmonary function is deranged as well as upon the severity of the abnormality. The degree of hypoxemia, the presence or absence of carbon dioxide retention, and the effects of exercise and oxygen breathing on the hypoxemia provide information about the nature and extent of the underlying abnormality of pulmonary function. This information may be of value in the differential diagnosis and treatment of patients with pulmonary disease. In addition, knowledge of the degree of hypoxemia which may result from impairment of each function of the lung provides insight into the relative severity of the hypoxemia that may result from a given disease. For example, a 50 per cent right-to-left shunt in the lung results in serious hypoxemia, whereas 50 per cent reduction of the diffusing capacity of the lung has little effect on the arterial oxygen saturation.

The accompanying classification of hypoxemia (Table 1) provides a useful framework for a discussion of this subject and of its treatment. Hypotonic hypoxemia refers to situations in which the arterial oxygen tension, and thus the arterial oxygen content, is subnormal; isotonic hypoxemia refers to situations in which the arterial oxygen content is reduced but the arterial oxygen tension is normal. Tissue anoxia also results from the actions of various poisons on cellular enzyme systems, so-called histotoxic anoxia, and in diseases in which circulatory impairment exists (occlusive arterial disease and cardiac failure), but these abnormalities are beyond the scope of the present discussion.

Hypotonic hypoxemia, which can be produced in normal subjects by reducing the inspired oxygen tension, occurs when the alveolar ventilation is diminished relative to metabolic needs, when the distribution of inspired air and of blood flowing through the lungs is not homogeneous, and when

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there is impaired diffusion of oxygen across the alveolar membrane. Each of these abnormal situations will be discussed separately.

Diminished alveolar ventilation (relative to metabolic needs) is characterized physiologically by elevated alveolar, and thus arterial, carbon dioxide tension, as defined by the following equation: alveolar ventilation $= \frac{CO_2 \text{ produced/ minute} \times (P_B - 40).*}{\text{alveolar carbon dioxide tension}}$ The relationship between the arterial

TABLE 1. HYPOXEMIA

- I. Hypotonic
  - A. Diminished alveolar ventilation relative to metabolism
    1. Diminished total ventilation
    2. Increased work of breathing
    3. Increased dead space
  - B. Impaired distribution of blood and/or gas in the lung
    1. Right-to-left shunt
       (a) Cardiac
       (b) Pulmonary
           (1) Anatomical
           (2) Functional
    2. Inhomogeneity of ventilation and perfusion
  - C. Impaired diffusion
    1. Reduced alveolar capillary surface area
    2. Increased thickness of aveolar membrane
- II. Isotonic
  - A. Reduced hemoglobin concentration
  - B. Abnormal hemoglobin

oxygen saturation and the arterial carbon dioxide tension when alveolar hypoventilation is the only functional abnormality is depicted in Figure 1, which is drawn for a subject with a respiratory quotient of .70, breathing ambient air with a partial pressure of oxygen of 150 mm Hg. The arterial oxygen tension was calculated with the use of the $O_2 - CO_2$ diagram,[9] and the oxygen saturation was determined from a standard oxygen dissociation curve. It is apparent that, as CO_2 retention develops, hypoxemia progresses at an increasingly rapid rate. It is also apparent that significant hypoxemia

* This expression represents the total pressure of gases in the respiratory tract, barometric pressure minus the vapor pressure of water at body temperature, and is necessary to permit use of alveolar carbon dioxide tension rather than fraction in the alveolar ventilation equation.

only begins to appear when the arterial carbon dioxide tension rises to fairly high levels — over 60 mm Hg.

Hypoxemia of this sort may be caused by increased metabolism without a normal increase in ventilation or by diminished alveolar ventilation. Since the alveolar ventilation equals the total ventilation minus that of the dead space, diminished alveolar ventilation may result either from a reduction of the former or from an increase of the latter (without a compensating increase of the total ventilation). The latter mechanism operates to a minor extent in pulmonary emphysema and to a major extent when a large artificial dead space, in the form of tubing between the patient's lips and the soda lime cannister, is added to a patient for the administration of anesthetic agents. Treatment consists of increases of the alveolar ventilation, by increase of total ventilation or by diminution of the dead space. The ventilatory needs of a patient during anesthesia or in a mechanical respirator may be predicted accurately by calculating the alveolar ventilation necessary to maintain the normal arterial CO_2 tension, measuring or assuming a normal dead space, and then calculating the required total ventilation.[8]

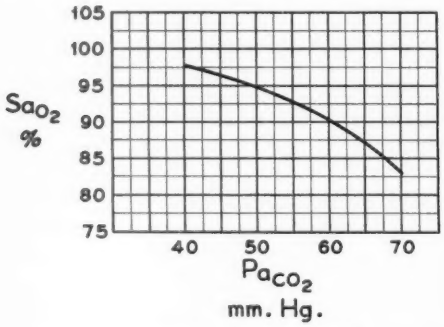

FIG. 1. The relationship between arterial oxygen saturation ($S_{a_{O_2}}$) and arterial carbon dioxide tension ($P_{a_{O_2}}$) in alveolar hypoventilation. Hypoxemia only occurs when appreciable CO_2 retention is present.

In pulmonary emphysema, inadequate alveolar ventilation may result from the fact that, in addition to a large physiological dead space, the work of breathing is increased to such an extent that the oxygen requirements of the respiratory muscles exceed the ability of the patient to provide adequate alveolar ventilation, so that hyperventilation may actually increase the arterial CO_2 tension.[19] As a result, treatment should include relief of the airway obstruction by means of bronchodilators and increase of the total ventilation by a positive-pressure breathing appliance or, in advanced cases, by a respirator. It has been repeatedly pointed out that the hypoxemia of these patients may be relieved by oxygen breathing, but that this has no effect on the arterial carbon dioxide tension and may indeed depress respiration, so that CO_2 retention will become more severe and deleterious.

Poor distribution of blood and gas flows to the lungs may take two forms —an actual effective shunt in which mixed venous blood flows directly into the arterial system without exposure to ventilated alveoli, and a lesser degree of impairment such that some areas of lung receive different amounts of blood and gas. The first abnormality, in which hypoxemia is the result of venous admixture with arterialized blood, is not accompanied by significant carbon dioxide retention. Figure 2 depicts the effect of varying

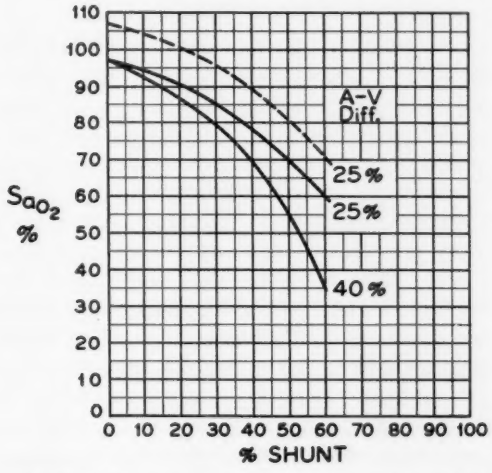

FIG. 2. The relationship between arterial oxygen saturation (S_{aO_2}) and per cent right-to-left shunt when room air (————) and when oxygen (— — — —) is the inspiratory gas mixture. Room-air curves for two arteriovenous oxygen differences are plotted.

sizes of shunt on the arterial oxygen saturation, calculated from a standard mixing formula.[17] The solid lines represent the effect of varying degrees of shunt on the oxygen saturation when room air is breathed and, in the presence of a normal resting arteriovenous difference (25 per cent), a large shunt is required to produce significant hypoxemia. When the arteriovenous difference widens, as it does during exercise or in resting individuals with diminished cardiac output, the same degree of shunting results in more hypoxemia. Inspiration of oxygen results in some increase of the arterial oxygen saturation, and this effect is shown quantitatively by the dotted line. The general relationship between oxygen saturation and per cent shunt is the same as when room air is breathed, but the level of oxygen saturation for a given shunt is elevated by virtue of the fact that the blood leaving the

well-ventilated lung capillaries is saturated to 107 per cent relative to the saturation of blood exposed to room air. Under these circumstances, a very large shunt is necessary to produce significant hypoxemia and one may, by measuring the arterial oxygen saturation during inspiration of room air and of oxygen, assess the relative contributions of a shunt and other factors in the genesis of the hypoxemia. Oxygen breathing eliminates hypoxemia due to all causes other than a right-to-left shunt so that hypoxemia persisting during oxygen breathing may be attributed to a shunt, the size of which may be readily estimated.

Hypoxemia due to a right-to-left shunt may occur in congenital anomalies, cardiac or pulmonary (pulmonary arteriovenous fistula), or in acquired disease in which blood flows through nonfunctioning pulmonary tissue. This is the case in patients with atelectasis, in whom there is often surprisingly little hypoxemia despite the presence of major airway obstruction.[1] Whether this is related to mechanical collapse of blood vessels in the atelectatic lung or to reflex vasospasm is unknown.[2] In treating atelectasis, it is important to realize that large pressures are required to open up collapsed lung (12 cm. of water or more) owing to the surface tension existing in the collapsed lobule.[5] It is also of interest to point out that atelectasis of a lobule does not result in complete absence of ventilation, owing to the presence of collateral ventilation through the alveolar walls,[14] but that atelectasis of a lobe results in total absence of ventilation since no communication exists between adjacent lobes. An interesting example of hypoxemia due to an effective right-to-left shunt is pulmonary edema, in which the shunt appears to result from the fact that blood circulates through areas of lung to which ventilation has been obstructed because foam and edema fluid occlude the small airways.[15] Successful treatment depends upon relief of the underlying abnormality responsible for the accumulation of edema fluid in the lung, and oxygen inhalation may have little effect on the hypoxemia.

The other type of impaired distribution of blood and gas in the lung which may result in hypoxemia occurs when ventilation and blood are not distributed evenly throughout the lung. This situation, occurring in the normal subject to some extent and in the experimental animal, particularly after collapse of the lung,[16] is due to the fact that the oxygen dissociation curve is not linear; when the subject breathes oxygen, the hypoxemia disappears. This type of abnormality may be accompanied by slight retention of carbon dioxide and is probably the major reason for the hypoxemia that is present in pulmonary emphysema. Severinghaus has devised a method, based on alveolar-arterial CO_2 differences, for calculating the "alveolar" dead space which is a function of this distribution abnormality,[18] and Farhi

and Rahn have shown how uneven distribution of blood and gas flows in the lung may result in hypoxemia.[4]

Impairment of diffusion of oxygen across the alveolar membrane occurs after extensive reduction of the alveolar capillary surface area available for gas exchange (pulmonary emphysema,[8] resection of lung tissue,[19] or pulmonary embolization[18]), and in diseases of the lungs in which there is marked thickening of the alveolar membrane ("alveolar capillary block").[7] In the

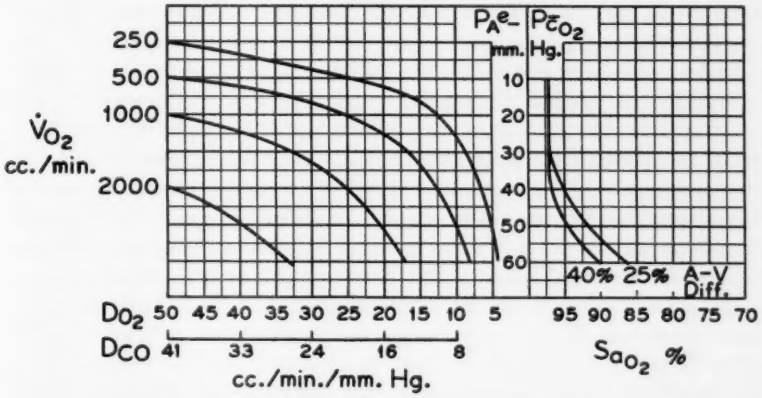

FIG. 3. The relationship between oxygen diffusing capacity of the lungs (D_{O_2}) and arterial oxygen saturation (Sa_{O_2}) under conditions of variable oxygen consumption (V_{O_2}) and arteriovenous oxygen difference. The ordinate, $P_A^{\bar{c}}$-Pc_{O_2}, is the difference of oxygen tension between the alveolar air and the mean capillary blood. To determine the arterial oxygen saturation, find the intersection of the diffusing capacity with the measured or assumed isopleth of oxygen consumption on the left-hand graph and carry over to the same ordinate on the right-hand graph where it intersects with the measured or assumed arterio-venous oxygen difference. Read the arterial oxygen saturation on the abscissa at this point.

latter case, there are generally some alveoli in which no diffusion of gas occurs, resulting in the presence of a variable right-to-left shunt. Since carbon dioxide is 20 to 30 times as soluble in blood and tissues (and, hence as "diffusible" from alveolar air to blood) as oxygen, impaired diffusion rarely results in carbon dioxide retention but may result in hypoxemia, particularly during exercise when oxygen requirements are increased and the limited diffusing capacity of the lung does not permit diffusion of adequate amounts of oxygen across the alveolar membrane.

The effects of a reduction of the diffusing capacity of the lung on the arterial oxygen saturation are quantitatively depicted in Figure 3. Since the

oxygen saturation, with respect to diffusion, is dependent upon the oxygen consumption, diffusing capacity, and the arteriovenous oxygen difference, a double graph is necessary to depict these relationships. The left-hand graph represents isopleths of oxygen consumption, each point being the product of the corresponding diffusing capacity and the difference of oxygen tension between the alveolar air and the mean capillary blood. The arterial oxygen tension is a function of the alveolar-mean capillary oxygen tension difference and the arterio-venous oxygen difference, and the right-hand graph was constructed from the line charts developed by Riley.[11] Oxygen tension was converted to saturation from a normal oxygen dissociation curve assuming a CO_2 tension of 40 mm Hg. A number of facts emerge from a consideration of this graph. For a normal resting oxygen consumption (250 cc./min.), the diffusing capacity must be greatly reduced to approach levels of alveolar-mean capillary oxygen tension difference which result in hypoxemia, even in the presence of a small arteriovenous oxygen difference. Thus, when the diffusing capacity is 10 cc. of oxygen per minute per millimeter of mercury, the mean gradient is 25 mm Hg and, for a normal arteriovenous difference, the arterial oxygen saturation is over 96 per cent. During muscular exercise, if the oxygen consumption increases to 500 cc. per minute, the arterial oxygen saturation would fall to 93 per cent if the arteriovenous difference did not change. However, one would anticipate an increase of the arteriovenous difference which would mean that the oxygen saturation would rise towards normal.

Although there must be considerable reduction of the diffusing capacity before there is any effect on the arterial oxygen saturation, beyond a critical level, hypoxemia develops at an increasingly rapid rate. Thus, a large portion of the lung may be resected without change in the arterial oxygen saturation, whereas removal of further lung tissue results in the sudden appearance of severe hypoxemia.[19] In the same way, several aliquots of glass beads may be injected into the veins of a dog to produce pulmonary emboli without a fall of arterial oxygen saturation, whereas one more injection results in severe hypoxemia.[18] Hypoxemia only appears when there is considerable reduction of the diffusing capacity, but once hypoxemia is present because of a diffusion limitation, further reduction of the diffusing capacity results in accentuation of the hypoxemia at an increasingly rapid rate.

Hypoxemia due to impaired diffusion is completely alleviated by the inspiration of oxygen, since the rate of diffusion of oxygen is directly dependent upon the pressure head of oxygen available for diffusion, and inspiration of 100 per cent oxygen increases this pressure head some six to eight fold. Conversely, reduction of the alveolar oxygen tension by ascent

to high altitudes or by inspiration of low-oxygen mixtures will greatly reduce the rate at which oxygen can pass across the alveolar membrane and may result in fatal hypoxemia. Thus, patients with a diffusion impairment must be cautioned against exposure to high altitudes but may achieve satisfactory improvement of the arterial oxygen saturation by inspiration of oxygen.

Isotonic hypoxemia results when the arterial oxygen tension is normal but the capacity of the blood for carrying oxygen is reduced. This situation occurs in anemia, when there is a decreased concentration of hemoglobin available for combination with oxygen, in methemoglobinemia, when the hemoglobin is altered so that it cannot combine with oxygen, and in carbon monoxide poisoning. The latter situation is unique in that, in addition to combining with hemoglobin to form carbon monoxide hemoglobin which will not combine with oxygen, the presence of carbon monoxide seems to change the characteristics of the remaining hemoglobin so that oxygen release to the tissues is impaired.[12] Obviously, treatment of this type of hypoxemia is dependent upon reconversion of carbon monoxide hemoglobin to normal hemoglobin, and carbon monoxide poisoning is treated by inhalation of oxygen. This promotes the formation of oxyhemoglobin rather than carbon monoxide hemoglobin, since the relative proportions of the two are directly dependent upon the ratio of the partial pressure of oxygen to the partial pressure of carbon monoxide in the blood.

Each type of abnormality has been discussed separately, but it must be borne in mind that hypoxemia is usually the result of impairment of more than one aspect of pulmonary function. However, it is useful to understand the quantitative relationships that exist between each type of functional abnormality and the resultant hypoxemia.

It is important to realize that hypoxemia and cyanosis are not synonymous. Cyanosis is a function of the absolute amount of reduced hemoglobin in the capillaries and of the quality and content of tissue between the capillaries and the observer's eyes. The degree of polycythemia becomes a direct factor in the intensity of cyanosis, irrespective of the arterial oxygen saturation. The rate of speed at which blood passes through the tissues, and thus becomes deprived of oxygen, is another important factor. These relationships have been quantitatively and lucidly reviewed by Lundsgaard and Van Slyke.[8]

SUMMARY

The mechanisms responsible for hypoxemia in patients and the clinical situations in which the various types of hypoxemia occur have been re-

viewed. The effects of reduced ventilation, right-to-left shunts, and impaired diffusion on the arterial oxygen saturation have been quantitatively depicted. Treatment of hypoxemia, based upon physiological considerations, has been outlined. Some of the adjustments that occur in pulmonary disease to minimize the development of hypoxemia, such as collateral respiration and secondary reduction of blood flow to nonventilated areas of lung, have been mentioned as well.

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EXPERIMENTAL PYELONEPHRITIS

II. Effect of Partial Ureteral Obstruction on the Course of Bacterial Infection in the Kidney of the Rat and the Rabbit†

In order to induce experimental pyelonephritis with bacteria of the coliform group it has been found necessary to injure the kidney in some way before introducing the bacteria. Ligation of one ureter has been the method employed most commonly.[4, 6, 7] This has the disadvantage of causing serious progressive injury to the kidney, eventually converting it into a thin-walled sac, which renders difficult a study of the subtle changes of early infection or of the natural course of the acute pyogenic infection. Mechanical injury, another procedure which makes the kidney susceptible to infection, involves the production of areas of hemorrhage and engorgement of the blood vessels.[1, 2] The undesirable features of this are the lack of resemblance to the pathogenesis of human pyelonephritis and the difficulty of distinguishing between the effects of early infection and those of the preceding mechanical trauma. It seemed desirable, therefore, to work with an experimental model in which there was partial ureteral obstruction, compatible with prolonged survival and function of the renal parenchyma, resembling a condition which commonly predisposes to the occurrence of pyelonephritis in man. Such a model has been developed by employing local irradiation of the ureter.[5] The present communication describes the effect of this form of partial ureteral obstruction on the susceptibility of the kidney of the rat and the rabbit to bacterial infection.

MATERIALS AND METHODS

Except where specified the methods and materials employed in this study were the same as those described in the preceding article in this series.[4]

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Irradiation of the ureter. Animals were anesthetized with pentobarbital sodium (10-12 mg. intraperitoneally in the rat, 20-25 mg. intravenously in the rabbit). After removing the hair of the left flank with electric clippers and cleansing the operative site with 70 per cent ethyl alcohol, an incision was made just below and parallel to the left lower rib margin. A small segment of ureter, approximately 2-3 cm. from the ureteropelvic junction, was freed from surrounding tissue and supported by a smooth, rounded wooden applicator stick. A strontium^{90} applicator (Tracerlab) was then placed against the ureter with gentle pressure for the required length of time. This applicator contains 100 millicuries of strontium^{90} uniformly distributed in a circular area with an active diameter of 5 mm. It is a pure beta particle emitter with an output of 102.6 rep (roentgen equivalent physical) per second at the surface. After desired irradiation had been given, applicator and wooden stick were removed and ureter returned to its normal position. The peritoneum was approximated with interrupted cotton sutures and skin wound closed with metal clips.

It was found that in the case of the rat a uniform degree of incomplete ureteral obstruction followed exposure to 700 rep. Some rats which received 1000 rep were also studied. In rabbits, exposure of the ureter to 6000 rep resulted in incomplete obstruction with moderate hydronephrosis. Irradiation with 4000 rep was associated with distention of the proximal segment of the ureter but only slight enlargement of the kidney.

Intravenous pyelograms. After induction of anesthesia with pentobarbital sodium, 50 per cent solution of sodium 3,5-diacetamide-2,4,6-triiodobenzoate (Hypaque, Winthrop Laboratories) was injected intravenously (2 ml. in the rat, 10 ml. in the rabbit). Pyelograms were obtained 20-25 minutes after injection in the rat and 5-8 minutes afterward in the rabbit. The technique for exposing the films was 31 KVP (rat) and 46 KVP (rabbit) at 30 milliampere-seconds and 72-inch distance, using par speed intensifying screens.

Examination of kidneys. With the animal under anesthesia the abdomen was opened and the kidneys examined. They were then removed; one-half of each was used for enumeration of bacterial content, the other half placed in 10 per cent neutral formalin for fixation, after which sections were cut and stained with hematoxylin and eosin.

EXPERIMENTAL

Effect of partial ureteral obstruction on susceptibility of the kidney to infection. (a) *In the rat.* Partial ureteral obstruction was induced in two groups of rats by ureteral irradiation. The presence of obstruction was verified in each one by pyelography 1 or 2 days prior to inoculation with bacteria. One group of 14 received 0.5 ml. of a 4-hour broth culture of *E. coli* (150 to 200 million bacteria) intravenously on the 7th day following ureteral irradiation; the other group of 15 received it on the 14th day. The kidneys were removed one week after the bacterial inoculation, homogenized in a glass grinder, and cultured quantitatively.

Three of 14 obstructed kidneys in the first group of rats were found to be heavily infected, i.e., to contain 10^5 to 10^7 bacilli. In the other group, 3 of 15 obstructed kidneys were found to be infected. All of the contralateral,

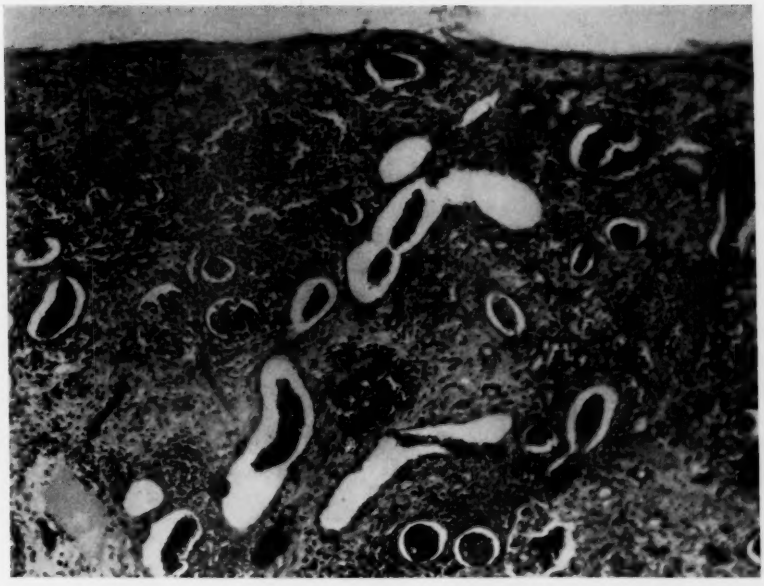

FIG. 1. Acute pyelonephritis in the cortical zone of the partially obstructed kidney of a rabbit. x135

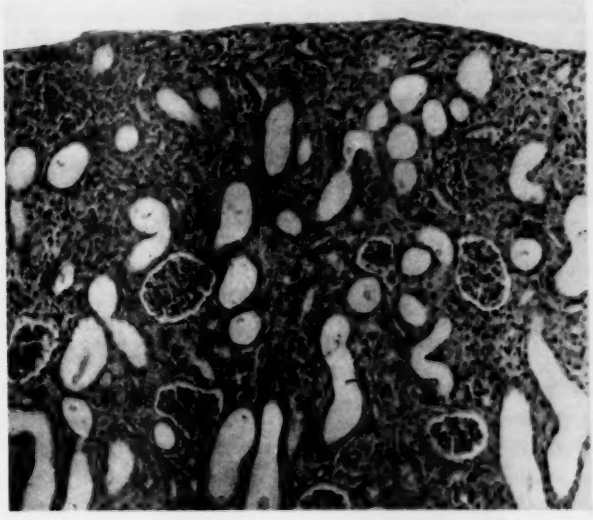

FIG. 2. Cortical zone of one of the rabbit kidneys which did not become infected following intravenous inoculation of bacteria. Tubular dilatation is conspicuous. x135

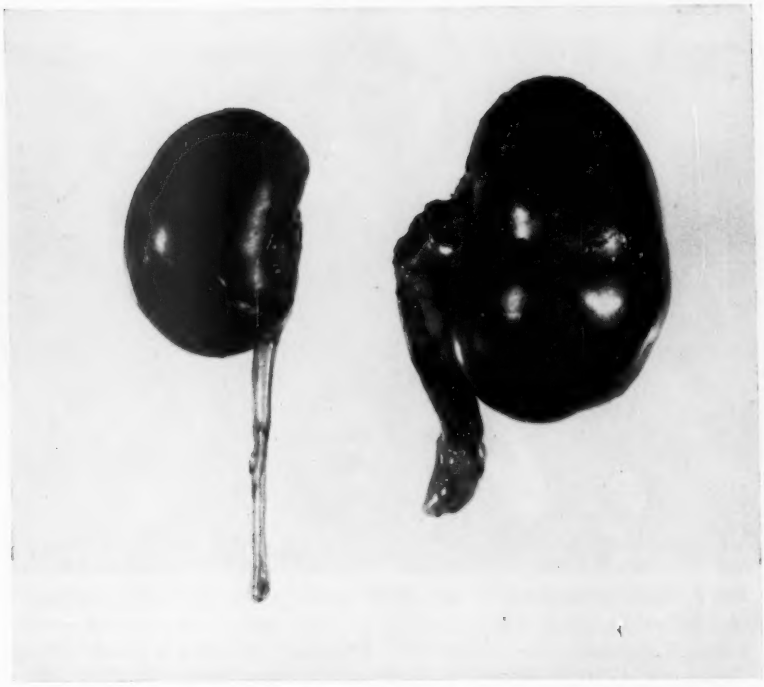

FIG. 3. Appearance of kidneys of a rabbit 3 weeks after irradiation of the left ureter. Despite the hydronephrosis, the kidney did not become infected following intravenous inoculation of bacteria.

FIG. 4. Intravenous pyelogram in a rat. Normal pelvic outline and proximal ureter on right of photograph; severe dilatation of kidney pelvis and proximal ureter on the left (irradiated) side.

unobstructed kidneys in the two groups of animals were uninfected, i.e., either there was no growth or the kidneys contained fewer than 10^2 bacteria, a quantity which might be expected to persist in a normal organ a few days after intravenous injection of this inoculum.[4]

In view of the low incidence of infection in the above two groups of animals, a third group was irradiated, this time with a slightly heavier dose, 1000 rep. After an interval of two weeks, the 10 animals were examined by pyelography and all were found to have hydronephrosis but good function, as judged by dye excretion. They were then given the same intravenous inoculation of colon bacilli. In addition, 4 rats with ligated ureters were injected with colon bacilli at the same time. One week later all 4 animals subjected to ligation showed heavily infected kidneys but only 2 of the 10 with partial ureteral obstruction had become infected.

(b) *In the rabbit.* Because of the low incidence of infection following intravenous inoculation of colon bacilli in rats with partial ureteral obstruction, it was decided to conduct similar studies in another animal. Seventeen rabbits received irradiation of 6,000 rep to the left ureter and 7 were given 4,000 rep. Two weeks later the same inoculum of *E. coli* was injected intravenously. In 3 of the former and 2 of the latter group, bacteriological and histological evidence of pyelonephritis was found. Complete ureteral ligation was performed in 2 animals; both of these rabbits had heavy infection when examined one week after the intravenous injection of bacteria. Figure 1 shows the microscopic appearance of early pyelonephritis in one of the rabbits with partial obstruction which became infected.

The remaining 19 irradiated animals had ureteral obstruction and hydronephrosis which did not differ discernibly from those which became infected. Gross and histological appearances are shown in Figures 2 and 3.

Partial ureteral obstruction and induced staphylococcal infection. Because of the low incidence of infection following intravenous inoculation of colon bacilli in rats and rabbits, some experiments were carried out in rats employing a strain of staphylococcus which had been isolated from a "spontaneous" infection in a rat with ligated ureter. This organism was hemolytic but coagulase-negative and had been maintained in the laboratory by weekly transfers on nutrient agar. Fourteen rats were treated with 700 rep to the left ureter and 2 weeks later were given 0.5 ml. of 4-hour culture, containing approximately 200,000,000 organisms. Four of the 14 animals, when examined 5 days later, showed evidence of staphylococcal infection of the kidney. This incidence is similar to that already obtained with the strain of *E. coli.*

DISCUSSION

The comparatively low incidence of pyelonephritis in kidneys which had been subjected to partial ureteral obstruction was an unexpected finding. Pyelonephritis in human beings is known to occur with much greater frequency when there is partial obstruction to free flow of urine, as by tumor, stone, or developmental defect. In these experiments, although conspicuous hydronephrosis had been caused, and bacteria had then been deliberately introduced, only about 1 in 5 of the animals developed infection in the obstructed kidney. This is in contrast with the effect of ureteral ligation, where infection had been found to result in nearly every instance, using a similar infectious inoculum. Moreover, other workers have found that complete ureteral obstruction for only a few minutes or a few hours may cause the kidney to be highly susceptible to infection.[8, 9]

There is no obvious explanation for the present findings. Comparison of the pyelograms done prior to the bacterial inoculation did not reveal a difference in the extent of hydronephrosis in the two groups. Certainly there was no sign of complete ureteral obstruction in the animals which became infected, although the possibility cannot be excluded that transient periods of complete obstruction, comparable to those in the experiments of others just cited, may have existed. This aspect of our findings, however, seems of less significance than the fact that infection failed to develop in a high proportion of animals with severe hydronephrosis.

The question may be asked whether the different effect of complete and partial ureteral obstruction may not be a matter of interference with excretion of organisms, i.e., that with partial obstruction bacteria are discharged into the ureteral lumen and carried away, whereas in complete obstruction they cannot be eliminated and hence multiply locally. In view of the fact that these bacteria do not appear in the urine of animals with normal kidneys following intravenous inoculation, it seems unlikely that this is the decisive factor.

Stagnation of urine flow is frequently cited as the factor which renders the urinary tract susceptible to infection in obstructive disease. The results obtained here do not indicate that this is of first importance, since the extensive hydro-ureter and hydronephrosis obtained by this method would prolong considerably the rate of passage of urine and bacteria through the upper urinary tract.

Other explanations can be suggested to account for the difference in effects of complete and partial obstruction on the susceptibility of the kidney to coliform bacterial infection. The chemical alterations which occur in the kidney substance in the presence of sudden and complete ureteral obstruc-

tion may favor bacterial growth or hinder antibacterial actions. With chronic, partial obstruction, alterations in lymph flow may develop to a degree not achieved with abrupt complete obstructions. Similarly, there may be differences in blood flow through the kidney with the two types of preparation. Although increases in hydrostatic pressure within the kidney obviously accompany both kinds of obstruction, there may be considerable differences in the two procedures.

SUMMARY

A study has been made of the effect of partial ureteral obstruction on the susceptibility of the kidney to infection. The method involved direct irradiation of the ureter of rats and rabbits. The presence of significant obstruction was verified in each animal by intravenous pyelography prior to bacterial injection. The bacteria employed to induce infection were *E. coli* and a strain of *Staph. aureus* which was hemolytic and coagulase-negative. Bacterial inoculations were given 1 week or 2 weeks after the irradiation.

Infection occurred in only about 20 per cent of rats or rabbits with incomplete occlusion of one ureter. This is in contrast with the effect of complete ureteral obstruction, which, with the same bacterial inoculum, causes infection almost without exception.

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EVENTS

GROVER F. POWERS LECTURE

October 17, 1957

PUBLIC RESPONSIBILITY FOR THE HEALTH AND WELFARE OF CHILDREN. By Martha Eliot, Professor of Maternal and Child Health, Harvard University School of Public Health, Boston, Massachusetts.

Public responsibility for the health and welfare of children is the responsibility that must be assumed by all the people as a whole. Only as citizens develop this sense of responsibility can we as a nation make sure that all children have a good chance to grow and develop normally, to have a happy family life with adequate food, shelter, clothing and medical care, to be protected by law from exploitation, neglect, and economic want, and to be provided with a substitute family when their own family breaks down.

We have made great progress in our quest for the healthy development of the child since the middle thirties. But we have made less progress in our understanding of the emotional development of children and of child behavior and in applying what knowledge we have to the health and social care of children.

Great discrepancies still exist in the medical care and preventive health services for children in this country depending on where they live—in an isolated or semi-isolated rural area vs. a large metropolitan city—or on the income and size of their family.

Three areas of action in the pediatric and child health program are urgent:

First. The times call for a new look at our health and medical care programs for children. Our child health forces must be concentrated in extending child health supervision to all children—services that are comprehensive in scope and adequate in their physical, social, and mental health aspects, and that are made available wherever children receive care, singly or in groups in the offices of physician, in prenatal clinics, child health conferences, school health programs, community health centers, in hospitals, children's institutions, in child welfare programs.

One of our greatest assets in accomplishing this lies in new tools and new knowledge now available through research in the natural, behavioral, and social sciences.

Second. We are confronted with a great shortage of professional workers—physicians, nurses, teachers, social workers, and other workers in contact with parents and children—who are well versed in the total care of children, who understand the stages and nature of growth, the factors that influence it and how deviations from the usual may be met. How can we educate professional persons to qualify them to work more effectively with children? Is it possible to increase the number of workers while at the same time standards are raised and content broadened? These are typical of the questions we must answer for all professions in contact with children and their parents.

Only within recent decades has this scientific knowledge of human development been included as a part of general medical education. Present-day medical students can have many opportunities for child health supervision in child health conferences, school health programs, programs for the care of handicapped children, and in many children's institutions. But for the greater part of their supervised experience, these students should be assigned to ongoing health programs of well-staffed local public health departments. If students from schools of social work and schools of nursing could be assigned to the same centers, the learning experience for all would be richer.

Third. Research must be extended into many areas of child life if more knowledge is to be available with which pediatric care of sick and well children can be improved, our understanding of child behavior enriched, and more effective prevention and treatment of emotional disturbances be provided in childhood and adolescence.

The pediatrician, general practitioner, obstetrician, and other child health workers must epitomize the best philosophy of public responsibility for the health and welfare of children which on the one hand calls for service focused on the peculiar needs of each child and on the other demands of forward looking planning for all children on a community-wide basis, whether that community is a village, a neighborhood, a city, a state, the nation, or the world community of nations. The most important business in the world even for governments as well as for parents is the rearing of children.

CARDIOVASCULAR AND GASTROINTESTINAL STUDY UNITS

December 16, 1957

PORTAL HYPERTENSION. By Charles G. Child, III, Tufts College of Medicine, Boston, Massachusetts.

Portal hypertension is, for the most part, an inocuous condition provided that its one major complication, hemorrhage, is prevented. There are two groups of people with portal hypertension to be considered—those with esophageal varices who have never bled, and those who have had one or more episodes of bleeding. Survival figures show that after the first episode of hemetemesis 40 per cent of patients will be dead within one month and 70 per cent will die in one year. Yet, after a successful portal decompression operation, the vast majority of patients are protected from future hemorrhage. The mortality from such surgery varies with the condition of the patient and his hepatic function at the time of operation and ranges from 3 per cent to 30 per cent. In addition there is an average 15 per cent morbidity largely from recurrent amine intoxication.

The management of the patient with varices who has never bled is difficult to plan as there are no figures on which to base the incidence of first bleeding. This is primarily due to the fact that people with portal hypertension and good hepatic function without bleeding varices do not present themselves to a physician. To subject this patient to the mortality and

morbidity of a portal decompression procedure as a prophylactic measure cannot be fully justified although in those patients with ascites the reduction in portal venous pressure may contribute to improvement.

In either group of patients it is advisable to institute a medical rehabilitation program if the condition of the patient is poor. This may extend over six months to a year at which time liver function, serum, albumin, the response to a salt and a protein load, and the general physical condition can be evaluated optimally. Reduction in portal pressure can be marked with disappearance radiographically of varices or reduction in ascites. An end-to-side portacaval anastomosis has been found most satisfactory.

R. N.

YALE MEDICAL SOCIETY

January 13, 1958

ADRENALECTOMY AND CARBOHYDRATE METABOLISM. By William Winternitz, Departments of Physiology and Internal Medicine.

Following epinephrine administration adrenalectomized rats show a large net deficit of the body stores of carbohydrate due to the greater loss of muscle glycogen and the failure of accumulation of liver glycogen as compared to normal controls. The abnormal response is corrected by replacement with adrenal cortical extract. The co-existence of alloxan diabetes and adrenalectomy does not prevent the excessive carbohydrate disappearance characteristic of the adrenalectomized state. Evisceration, however, does abolish the differences in the response to epinephrine of normal and adrenalectomized animals. Adrenalectomized rats perfused with various carbohydrate precursors also show a larger utilization of carbohydrate than do normal controls.

The respiratory quotient of adrenalectomized animals given epinephrine is maintained at a high level for about three to four hours after epinephrine, while in the normal animal there is little change than that caused by the displacement of CO_2 by lactic acid. When $2C^{14}$ pyruvate is administered as a tracer following epinephrine, the adrenalectomized rat exhales more $C^{14}O_2$ in the ensuing four hours.

It is concluded that the adrenalectomized animal not only forms less but also utilizes more carbohydrate than does the normal control. The chief site of action is believed to be hepatic, and it is felt that the oxidative route of carbohydrate utilization is the one affected most by the presence or absence of the adrenal cortical hormone.

W. W.

January 13, 1958

AMNIOTIC FLUID EMBOLISM AND MATERNAL DEATH. By Harold D. Attwood, Research Fellow, Department of Pathology.

Amniotic fluid embolism is a histologically defined entity but a clinically obscure one. It was first described in 1926 by Ricardo Juvenal Meyer (*Brazil Med.*, 1926, *2*, 301), and since then numerous case reports have

appeared in the literature. Microscopically, it is characterized by the presence in the maternal pulmonary vessels of epithelial squames and lanugo hair from the amniotic fluid, fat from the vernix caseosa, and mucin from meconium contamination of the amniotic fluid. This foetal material can also be isolated from blood taken from the inferior vena cava or right heart and, in a number of cases, has been demonstrated, histologically, in the myocardial, cerebral, renal, and uterine vessels. At autopsy, the only gross changes are cyanosis and a dilated right heart. The lungs show no abnormality visible to the naked eye.

In a histological study of the lungs from 119 pregnant, parturient, and puerperal women dying in two Scottish hospitals, trophoblastic giant cells were found in the maternal vessels in 44 per cent of the cases. Such trophoblastic emboli are not regarded as pathologically significant, but are probably normal concomitants of pregnancy and labour. In only two cases, however, could the presence of amniotic fluid debris within the maternal pulmonary vessels be demonstrated. In one of these cases, only a few squames and one strand of mucin could be seen; death had been the result of chloroform toxicity. In the other, the foetal debris was widespread in the maternal pulmonary vessels and could be demonstrated in sections taken from 15 blocks of lung tissue. In this case, the woman had died suddenly during an apparently normal labour following an apparently normal pregnancy. Amniotic fluid embolism was the only pathological finding.

The presence of the foetal debris in the maternal pulmonary vessels can be overlooked in routinely stained (haematoxylin and eosin) sections. The application of special staining techniques can make the diagnosis easier and, hence, more certain. The fuchsinophilia of the epithelial squames can be best demonstrated in the picro-Mallory preparations. The large amount of foetal debris that may be present in the materal pulmonary vessels is best demonstrated by a combined stain for the mucin and epithelial squames. A combination of Alcian green for the mucin with the phloxin tartrazine method to demonstrate the squames has been found to be most useful.

The cause of death in cases of amniotic fluid embolism is a matter of some controversy. On the one hand it is believed that the foreign material may be toxic to the mother and death may result from an "anaphylactoid reaction." Others believe that obstruction of the maternal pulmonary vessels by foetal debris may be the important lethal factor. In experiments to study the cause of death, mice were injected intravenously with amniotic fluid or meconium suspensions. A constant injection technique of 1 ml. in one minute was used. It was found that the number of animals dying as a result of the injection was directly related to the amount of suspended material in the fluid. When filtered fluid was injected, no animals died. Experiments are currently being carried out on dogs to measure the changes in pulmonary artery pressure following the intravenous injection of amniotic fluid and meconium suspensions.

D. W. E. S.

BOTANY SEMINAR

January 14, 1958

THE MECHANISM OF GENETIC RECOMBINATION THROUGH TRANSDUCTION IN *Samonella*. By M. Demerec, Carnegie Institute of Washington, Cold Spring Harbor, Long Island, New York.

One cystine and four tryptophane markers were used in preliminary experiments in *Salmonella* in order to shed some light on the mechanism of induction. The five loci are linked (probably since the tryptophane loci are functionally related) in a previously determined order and are always carried on a single transducing segment. By plating on indole and cystine and by further transduction experiments, 26 of the 31 possible recombinants from reciprocal transductions have been recovered and identified. The results were found not to be influenced by type of marker nor by whether a given allele was carried on a bacterial chromosome or on a transduced fragment, but were similar to crossover results. Crossover frequency data permit calculation of the distances between loci relative to over-all length of fragment. Since single crossovers would produce long inviable deficiencies, they cannot be observed directly, but calculations from data indicate that they may take place. Double crossover frequencies are within the expected values. The existence of negative interference may be inferred from the occurrence of quadruples and of a single sextuple crossover in excess of expectation. The latter is at present unique in genetic literature. An estimate of the base line is impossible since all the phages do not transduce; the data presented are taken to indicate, however, that 50 per cent of the fragments enter into transduction.

These data suggest a possible mechanism for transduction: during formation of new chromosomal material, the reproducing element may choose either the region of the bacterial chromosome or the phage-transduced fragment as a template for the chromosomal segment involved. Each copy choice influences the succeeding one nonrandomly.

G. E. W. 3RD

ZOOLOGICAL JOURNAL CLUB

January 15, 1958

ONOTOGENY OF ENZYMATIC ACTIVITY. By Clement L. Markert, Professor of Biology, Johns Hopkins University, Baltimore, Maryland.

Electrophoresis combined with histochemical determination on cell extracts provides a technique for measuring esterase activity at various stages of growth. Cell extracts are placed on a starch gell strip and electrophoresed at 6 volts/cm. and a pH of 8.6. Enzyme localization on the anodal strip is then analyzed by providing a reaction substrate and a diazonium salt dye. Various bands of reaction with the dye indicate specific enzyme activity; intensity gives a ratio of relative concentration. Substitution of substrate and use of different inhibitors permits specific enzyme analysis.

This method will work on any electrophoretically labile enzyme which may be histochemically analyzed.

Twenty esterases have been thus demonstrated in mouse liver. Various esterases in the liver, eye, and duodenum are added, and relative amounts of enzyme change during pre- and postpartum growth. Four patterns of change in enzyme activity with increase in age are found in various mouse tissues: gradual rise, logarithmic growth, high initial concentration with postpartum decline and adult increase, and a sudden appearance or increase at adulthood.

These data imply two methods for cellular differentiation: genetic makeup and cellular interaction. Mouse tissue culture extracts show a different electrophoretic pattern by this method which suggests that although genes are instrumental in cell differentiation, their presence is not sufficient for enzyme differentiation, but the environment of the cell during development is of great importance.

G. E. W. 3RD

February 12, 1958

CHEMORECEPTION AND THE REGULATION OF FEEDING IN THE BLOWFLY. By Vincent G. Dethier, Professor of Biology, John Hopkins University, Baltimore, Maryland.

Mechanisms regulating feeding behavior in the black blowfly *(Phormia regina)* indicate significant implications in relation to the concepts of vertebrate behavior.

Gustation occurs by means of chemoreceptive hairs localized primarily on the tarsi and labellum of the fly. Each hair is associated with three bipolar sensory neurones, one responding to "acceptable" stimuli (e.g. sucrose), another to "unacceptable" stimuli (e.g. NaCl), and the third to bending of the hair. Exposure of one hair to an acceptable solution will elicit a feeding response (proboscis extension) from a hungry animal. The threshold sugar concentration leading to a feeding response is an inverse function of the degree of starvation. A rise in response threshold is not a result of receptor cell adaptation; thus, threshold is centrally regulated.

In the alimentary tract, when the midgut becomes empty, stored food from the crop is transported to the oesophagus, thence to the midgut. The inhibition of feeding behavior is a result of the presence of fluid in the oesophagus. The central nervous system is informed of fluid presence via the recurrent nerve leading to the hyperpharyngeal ganglion, thereby causing adjustment of the response threshold. Flies with transected recurrent nerves were unable to stop feeding. Nothing is known of the oesophageal receptors involved.

These results are significant with respect to the concepts of vertebrate behavior. Thus, the *consummatory act* to cause cessation of feeding is neither a Lorenzian "drainage" of the nervous system, nor a fulfillment of a metabolic need, nor a function of a given amount of muscular activity; cessation of feeding is simply the result of inhibitory impulses from the fluid-filled foregut. *Drive* results in this case from the interaction of exter-

nal and internal sensory receptors and has nothing to do with endogenous central nervous activity. The feeding and satiation *centers* can perhaps be considered at the level of the synapse in the blowfly.

F. M. WILLIAMS

February 19, 1958

EVOLUTION IN TROPICAL FORESTS AND THE PROBLEM OF FAUNAL DOMINANCE. By Edward O. Wilson, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

The ant subfamily Ponerinae in Melanesia shows a three-step pattern of distribution: (1) Ants have invaded the islands mainly from the Oriental region, a few have entered from Australia; (2) they have diversified in the islands; (3) the parental stock has vanished from the mainland leaving relict forms in the islands. There has been little backcurrent so that Melanesia has been an evolutionary *cul de sac*. The greatest number of ant species is found living in rotting wood in the thin leaf litter on the floor of the tropical rain forest; fewer species are found in marginal areas. Species in the littoral, savannah, and monsoon forest (marginal areas) are chiefly from the first category, those in the montane rain forests are almost entirely of the second and third categories. Rain forest species are found in a distributional mosaic as an almost closed ecological system which must render direct colonization from the mainland difficult, if not impossible.

A possible hypothesis to explain this pattern of distribution is proposed. During a fluctuation peak in an Oriental rain forest, part of a mainland ant population expands into a marginal area. This portion may undergo genetic adaptation to the new environment. Some cross the water gap and, if adapted, may colonize insular marginal areas and spread. Once established, they must quickly readjust in order to re-enter the optimal rain forest, since the peripheral area is truly marginal for Ponerines. When back in the rain forest, they diversify, and may even reinvade the marginal area. In some cases, the parental population becomes extinct on the mainland.

G. E. W. 3RD

BOOK REVIEWS

THE LEUKEMIAS. ETIOLOGY, PATHOPHYSIOLOGY, AND TREATMENT. Henry Ford Hospital International Symposium. John W. Rebuck, Frank H. Bethell, Raymond W. Monto, Eds. New York, Academic Press, 1957. 711 pp. $13.00.

This book represents the proceedings of the highly successful International Leukemia Symposium held in Detroit in March of 1956. It is arranged under eight major headings dealing with such phases of leukemia as its submicroscopic morphology, transmissibility, immunology, and biochemistry. Each of the major headings embraces a number of individual papers, thirty-nine in total, as well as prepared comments and general discussions. Illustrations include graphs, tables, and photographs.

The photographs of Bessis's electron microscopic views of leukocytes are both beautiful and amazing. He has been able to prepare 800 microscopic sections of a single leukocyte. Structures, such as granulations, nucleoli, and Auer bodies stand out with great clarity. The identification, however, of blasts of different origin remains uncertain even with this advanced technique. Equally nice to see under phase contrast microscope are plasma cells removed from the spleen during active immunization. Their large antibody-producing mitochondria actually can be visualized.

Discussion by S. C. Finch on the antigenicity of different leukocytic fractions reveals that the best antileukocytic effects can be obtained when cytoplasm is used as the antigen. This appears to be one of the first instances where antigenicity is localized to a cell fraction. In this paper the possible treatment of leukemia with a potent cell-type specific antisera also is mentioned.

Studies with transplantable leukemias in rodents contribute especially to our knowledge of the role of filterable agents, cell fragments, and antigenic mutations in the etiology of leukemia. Among the interesting problems are the immunological aspects of "take" of a transplant and the question of biological unity or difference between the different manifestations of transplanted tumors. The introductory remarks reflect sobering thoughts on the subject, ". . . it should be remembered that leukemic grafts and animals bearing them are not exact analogs of either the growth or the hosts in spontaneous disease." Other studies on the etiology of leukemia show that ionizing radiation is the only well documented amongst the environmental factors in leukemogenesis.

The remaining presentations are highly specialized biochemical studies on leukemic cell metabolism. The important role of nucleic acid synthesis is pointed out along with many therapeutic agents aimed against this target. Antimetabolites and purine analogues act by interrupting the chain of events in normal nucleoprotein metabolism. However, the need for more selective agents is recognized by all.

The 60 pages of "round table discussion" on therapy give a dynamic exposition on the present status of treatment. The results of many years of personal trials and errors are presented here by the discussors. Although optimism and pessimism carry individuals to one or another direction, on the whole, members of the panel—Burchenal, Dameshek, Doan, Farber,

Gellhorn, Hill, Moeschlin, and Wintrobe—were in close agreement regarding the indications for and therapy of leukemia.

This book is almost an encyclopedia in this particular branch of hematology. The reader will be impressed by the ingenuity and the amount of information it contains. Another of its assets is that one obtains good insight into the character and type of work being undertaken in the leading laboratories and clinics in this field. It is most stimulating reading for students, investigators, and clinicians interested in hematology.

KATHERINE D. DETRE

USES OF EPIDEMIOLOGY. By J. N. Morris. Edinburgh and London, E. & S. Livingstone, Ltd., 1957. 136 pp. $5.00.

This small book, which can almost fit into one's pocket, is recommended by the reviewer for perusal by clinicians and medical students and others to whom this provocative title may appeal. Heretofore epidemiology has usually been regarded as a discipline useful primarily for those engaged in public health activities. One might ask then why physicians should be concerned with it, particularly since there is so much to read about in more familiar clinical fields. But one finds from this book that epidemiology is fast coming of age among the clinical disciplines.

Dr. Morris, Director of the Social Medicine Research Unit of the Medical Research Council, London Hospital, and at present (1957-58) Visiting Professor of Epidemiology at the Yale University School of Medicine, is singularly well equipped to expound this subject to a clinical audience as well as to public health workers. He points out that modern epidemiology is no longer a subject limited to infectious diseases, but is one which if properly applied completes the clinical picture in a great variety of diseases. He develops a philosophy in a few pages which leaves little doubt that physicians can gain considerable benefit from inquiries which can tell them what is happening to their patients, decade by decade, what is happening in their community, and how this compares with what has happened elsewhere. What, for instance, might be the effects of various ways of life on the prevalence of coronary heart disease, diabetes, and duodenal ulcer in London, or in the Bronx, in Harlem, or in India? Dr. Morris is in a strong position to develop these ideas, having spent many years in effective studies on the epidemiology of coronary occlusion and other diseases. He knows his subject, writes well in what might be termed a salty vein. For these and quite a number of other reasons the reviewer can heartily endorse this small book, which costs little, takes only a short time to read, and is well worth the price and effort.

JOHN R. PAUL

FROM STERILITY TO FERTILITY. By Elliot E. Philipp. New York, Philosophical Library, 1957. 120 pp. $4.75.

Although in certain details the British attitude toward infertility as described by Elliot Philipp in *From Sterility to Fertility* is somewhat

different from that in the United States, this short volume, prepared for the lay reader, would be of considerable value to those infertile couples who wish to know more about their childlessness.

Dr. Philipp has undertaken the always difficult problem of explaining a complex technical situation to readers unprepared by education or experience to absorb the implications of what he is trying to say. This is, of course, the curse of all "science writing," medical or otherwise, because the inevitable necessity for oversimplification unquestionably leaves the reader with a certain number of false impressions.

If Dr. Philipp is correct in his figures, infertility in England is apparently greater than it is here, occurring there in one couple out of six whereas ten per cent of involuntary infertility is the figure usually quoted in this country. There is an interesting chapter somewhat reminiscent of Frazer's *Golden Bough* concerning the history and folk lore of infertility as well as a very straightforward account of sexual techniques as they are related to reproduction.

I doubt very much if physicians in this country would consider either "over-keen-ness" or "excessive smoking" as a proven cause of infertility, or a "failure of the womb to grow fast enough" as being a cause of habitual abortion. These matters are somewhat incidental, however, and certainly do not detract from the value of the book. On the other hand, there is one comment which I hope will be corrected if the book goes to another edition, and that is the statement that 50 per cent of tubal plastic operations on the female are successful. This is a highly unrealistic and overoptimistic figure which is not borne out by facts either in this country or abroad.

The bibliography is most unfortunate in its selection of American titles. Two of the volumes referred to are out of print, the name of one of the publishers is spelled wrong, and with one or possibly two exceptions the texts quoted are not considered authoritative in this country.

It is also unfortunate that the illustrations are so amateurish technically and that the jacket and paper used in publishing the book is not more agreeable.

C. LEE BUXTON

THE BIOLOGICAL ACTION OF GROWTH SUBSTANCES. Symposia of the Society for Experimental Biology, No. II. New York, Academic Press, 1957. vii, 344 pp. $9.50.

The nineteen papers in this volume constitute a valuable survey of recent developments and approaches in the study of growth control by specific substances. As a botanist, this reviewer was more at home with the ten papers on plant growth, but there appears to be little difference in the high level of excellence throughout the collection. One of the major defects to be found in some recent symposium volumes is the inclusion of papers which, because of their relatively limited scope, might better remain in technical journals. None of the papers included here can fairly be so described, although several—notably F. G. Gregory and J. A. Veale's stimulating "A reassessment of the problem of apical dominance," which

demotes auxin to a minor role in the phenomenon—are primarily presentations of new experiments.

A majority of the papers provide comprehensive reviews of the work of one laboratory or investigator over a period of years. Thus, for example, H. Burström's "Auxin and the mechanism of root growth" or V. B. Wigglesworth's "The action of growth hormones in insects" enable the reader to follow the development of a field as viewed by a leading worker in it and in the context of a responsible and relevant survey of the literature. Similar remarks can be made about close to a dozen of the other articles, ranging from P. J. Randle's "The growth hormone of the pituitary gland and metabolic processes" to J. R. Raper's "Hormones and sexuality in lower plants." The latter is valuable not only for its lucid coverage of some very complex interactions, but also for its review of recent attempts to confirm the controversial work of F. Moewus, which "now appears suspect at best or spurious at worst."

Two pairs of papers deal with similar problems involving plant and animal materials. "The effects of some microbial metabolic products on plant growth," by P. W. Brian, and "Antibiotics and animal growth," by J. W. G. Porter, are both concerned with the growth-promoting properties of certain antibiotics, although the former article also reviews some effects of indoleacetic acid and the gibberellins. While it seems clear that the effects of antibiotics on animals are "primarily due to their antibacterial action," the effects on plant materials under aseptic conditions remain unexplained. Some of the metabolic changes involved in the origin of tumorous tissues are discussed in A. C. Braun's "A physiological study on the nature of autonomous growth in neoplastic plant cells" and in G. and E. Klein's "The evolution of independence from specific growth stimulation and inhibition in mammalian tumour-cell populations." The former leaves entirely open the question as to whether one master reaction may be affected by the tumour-inducing principle causing crown-gall in plants, while the latter suggests that, in animal cells, it seems most unlikely that any master cellular change is responsible for the independence of neoplastic cells.

While the observation that things are complex is a cliché in biological research, at least two papers demonstrate this lamentable truth in very elegant fashion. "Chemical regulation of growth and organ formation in plant tissues cultured in vitro," by F. Skoog and C. O. Miller, summarizes the work of Skoog's group on the interactions of auxins, purines, and other substances in controlling differentiation. "The study of growth factors in tissue culture" by J. D. Biggers, L. M. Minaldini, and M. Webb is an extensive discussion of the use of chemically defined media in studying the growth requirements of animal cells and of some of the pitfalls that may arise from an oversimplified approach to the subject.

Space does not allow individual mention of the other papers. The volume should be included in every biological reference library and is a highly desirable addition—depending on the availability of $9.50—to the personal collection of any biologist with physiological interests.

WILLIAM S. HILLMAN

THE PHYSIOLOGY OF FISHES. Volume 2, BEHAVIOR. Margaret E. Brown, Ed. New York, Academic Press Inc., 1957. xi, 526 pp. $14.00.

The second volume of the series *Physiology of Fishes* deals with a wide variety of subjects under the common heading of Behavior. This misleading title is unfortunate, but the contents are a very pleasant surprise. Included are chapters dealing with: nerve system (central and autonomic); various organs of special sense, such as the eye, acoustico-lateralis system, as well as the gustatory and olfactory senses; swim bladder; electric and luminous organs; pigments; color changes; physiological genetics; water quality requirements; behavior, including conditioned responses, ethological analysis, reproductive and parental behavior. Many of these subjects have lain fallow overly long and thus have sorely needed review by competent workers.

The material is well presented and often so superbly summarizes the field that the curious reader will be stimulated to pursue further the provocative ideas. Further, the material is not limited to academia but both explains and suggests practical methods to fisheries, biologists, and conservationists.

In the remarkable selection of the authors for the various chapters, the editor has given us an impressive list of authorities whose high caliber and precision of writing unquestionably make this volume among the best of the many dealing with fishes which have appeared in the last few years.

The severest criticism which may be applied to this work is, as indicated above, the unfortunate choice of the sub-title. This volume is enthusiastically recommended.

WALTER CHAVIN

THE FIGHT FOR FLUORIDATION. By Donald R. McNeil. New York, Oxford University Press, 1957. x, 241 pp. $5.00.

This is not a book for readers who have little knowledge of the amazing ramifications of the currently raging controversy over fluoridation of public water supplies. McNeil is not a scientist who attempts to settle the issue by weighing the arguments for and against it; his obvious purpose was to produce an entertaining, semihistorical account that would help win over converts for unquestioning acceptance of the novel public health measure.

As a contribution to the history of modern medicine (which must be conceived of as including dentistry as one of its subdivisions) the publication cannot be ignored completely by the serious student. It contains a great deal of information pertaining to early researches on the dental disfigurement termed "mottled enamel" and the subsequent incrimination of waterborne fluorine as an important etiological agent. The first two chapters are particularly good in this respect. However, only investigations conducted in the United States are described.

Of special value are the appended "Notes to the Text," which consist largely of references to unpublished private papers donated to the State Historical Society of Wisconsin. Of equal worth is the author's piecemeal portrayal of some of the leading actors in the drama of fluoridation promo-

tion—that of J. G. Frisch, who headed the "Wisconsin group," being a jarring revelation to most readers. "The Madison dentist was a man possessed," whose greatest interest lay "in the promotional rather than the scientific side of fluoridation," and whose emotional reactivity blinded him to any and all suggestions for moderation in dealing with the problem.

It is regrettable that the volume draws far more attention to the humorous side issues and strange personalities of the controversy than to the serious basic problems that it poses. "The Wisconsin group took fluoridation out of the scientific field and thrust it into the political arena." McNeil is amused by the spectacle and writes about it with the relish one would expect from a young news reporter. It is a safe prediction that his book will not help to thrust the controversy back where it belongs.

V. O. HURME

CHEMICAL ANTHROPOLOGY, A NEW APPROACH TO GROWTH IN CHILDREN. By Icie G. Macy and Harriet J. Kelly. Chicago, The University of Chicago Press, 1957. 149 pp. $3.75.

This book comes as another in the series of publications on the remarkable and valuable studies conducted, under the direction of Dr. Macy, on the nutrition and growth of children. However, while based on the data collected and presented in the three volumes of *Nutrition and Chemical Growth in Childhood,* it pioneers a new interdisciplinary approach whereby the tools of physical anthropology and physiology are applied to the previous essentially chemical studies. Their approach uses a new conceptualization of physical anthropology as a dynamic science which obtains and studies its measurements in terms of function and life. Or, as the authors state, "the scope of physical anthropology has been expanded and extended to include chemical growth and its relationship to physical growth and physiological function. We call this *chemical anthropology.*" They have reinterpreted their data in terms of this concept and evolved a protocol for analysis using it as a basis. This has considerably broadened the sphere of interest of the work so that where it formerly included mainly the nutritionist and biochemist, it now includes the physiologist, the pediatrician, and certainly the practicing physician.

A consideration of the soft tissues—muscle, neural, and organ—and the hard tissues is presented. Certain phases of nitrogen, calcium, and mineral metabolism are analyzed in relation to age, change of body size, maturation and body composition during the physiological adjustments and adaptations of childhood. For example, the nitrogen and calcium ingested and excreted are analyzed physico-chemically, and their interrelationships and functions are considered. Incremental increases of the entire body, organ systems, tissues, etc., are computed anatomically, chemically, radiologically, and through the use of highly specialized techniques and formulae of physical anthropology. In following through, one obtains a grasp of the metabolism of living protoplasm with respect to these minerals. Suggestions for more complete and other future analyses are given.

Chapters are also devoted to the "Metabolic Fate of Nutrients," "Body Composition," and to a general discussion of chemical anthropology. In addition there is an interesting chapter on the "Mosaic of Physical and Chemical Growth."

This small, well-written work presents a new approach to the highly important field of *in vivo* metabolism and physiology of the body. It is recommended not only to those interested in research along this line but also to the physician who will be rewarded with a new perspective of growth and metabolism.

N. R. C.

MITOCHONDRIA AND OTHER CYTOPLASMIC INCLUSIONS. Symposia of the Society for Experimental Biology, No. 10. New York, Academic Press, 1957. 198 pp. $9.50.

In the past decade, with the application of the techniques of electron microscopy and differential ultracentrifugation to the study of the internal organization of the cell, the elucidation of the anatomy and the biochemistry of subcellular particulate structures has become one of the most rapidly advancing fields of experimental biology. This rapid progress has not come without bringing with it a certain amount of confusion: for example, in nomenclature, in our knowledge about the degree of homogeneity of isolated particles, and in the relationship of these isolated particles to cellular structures visible *in situ*. This volume, the tenth in an excellent series, maintains the high standards set by its predecessors and does much to dispel confusion in a difficult field. As the name of the book implies, a great deal of attention is given to mitochondria. This symposium did not concern itself with microsomes or the endoplasmic reticulum.

If there are any serious criticisms to be made of this volume, they are the length of time which has been permitted to elapse between the time the symposium took place and the date of publication (20 months), and the lack of an index. In a field which is progressing as rapidly as this one, a shorter publication time would have been helpful.

The volume consists of a series of 11 papers read at a Symposium of the Society of Experimental Biology held at Oxford in September, 1955. The opening paper by J. R. Baker, later ones by A. J. Dalton and Marie D. Felix and by D. Lacy and C. E. Challice, discuss our present knowledge of the structures called the "Golgi complex." Both papers are excellent and do much to define clearly the problems involved both of nomenclature and of identification, in what is one of the more confusing aspects of present-day cytology. C. de Duve and A. Novikoff, in two outstanding papers which should be read by all workers in this field, discuss the problem of the biochemical and structural heterogeneity of isolated cell fractions. In spite of a good deal of evidence at hand, both authors agree that it is not yet possible to reach a decision as to whether the isolated subcellular fractions are, either biochemically or anatomically, homogeneous or heterogeneous in nature. The biochemical properties of mitochondria themselves are the subject of extremely informative and important papers by D. E. Green and by E. C. Slater. The former author discusses the evidence for

the view that the mitochondria are, rather than a "bag of enzymes," a highly organized structure in which enzymes are tightly bound in very specific positions to increase the efficiency of the many-step conversion of a substrate to CO_2 and H_2O and of the associated transport of electrons. The latter author discusses the biochemical and physical properties of muscle mitochondria and concludes that, with several exceptions, differences between these and liver mitochondria are minor. An important source of confusion in nomenclature (relation between sarcosomes and mitochondria) is cleared up in this paper.

Other interesting papers include a discussion of the subcellular structure of the snail amoebocyte by G. N. C. Crawford, of plant roots by J. Chazen and S. F. Jackson, and of a protozoan organism by J. T. Randall. An interesting paper by R. Barer and S. Joseph shows what can be done using phase-contrast and interference microscopy. This book can be highly recommended to all interested in this field; it might well be considered "required reading" to those actually working with cell particles.

MELVIN SIMPSON

THE CHRONICALLY ILL. By Joseph Fox. New York, Philosophical Library, 1957. 229 pp. $3.95.

The author imparts with empathy problems involved in the care of the patient with a prolonged illness. This book is an important addition to medical literature. In its entirety it is a compilation of much of the voluminous current thought and writing on this subject by a man who has had extensive experience. He is at his best when he deals with the aspects of care with which he is most familiar, i.e., the social, psychological, philosophical, and administrative. Provocative thoughts are presented in exploring the reactions of those who come in contact with and of those who avoid contact with the chronically ill.

Unfortunately, vague and at times improper medical clarifications along with typographical errors detract from the presentation of this material. These faults could have been avoided by adequate proofreading. At times statistics appear to be used excessively, but these can be skimmed over with minimal loss of impact. In the preface, reference is made to the fact that information in this book "should be of particular interest to the busy physician." More condensation and less repetition might have made that physician more receptive. Still, the busy physician, the student of medicine or social work, the teachers of these groups, and the trustees of private and public institutions concerned with patient care will benefit from reading this book. The above-mentioned shortcomings must not deter one from seeking and digesting the information Dr. Fox presents.

Referring again to his preface, the approach in this book "is first to deal with chronic disorders as they strike the individual, and only after they have been discussed in that setting are the problems of organizations, institutionalization, finance, and ethics discussed. This is not a manual on medical treatment."

ROBCLIFF V. JONES, JR.

RECENT PROGRESS IN HORMONE RESEARCH, Vol. XIII. Proceedings of the Laurentian Hormone Conference, 1956. G. Pincus, Ed. New York, Academic Press, 1957, viii, 646 pp., illus. $12.80.

This volume, a collection of papers presented at the Laurentian Hormone Conference, September 2-7, 1956, records the recent progress in five areas—(I) Neurohumoral-Endocrine Relationships: (a) serotonin, (b) CNS and metabolic processes, (c) CNS and anterior pituitary function, (d) hormones and rhythms; (II) Hormone Transport in Circulation: (a) thyroid hormones and protein in biological fluids, (b) binding of steroids to human plasma proteins; (III) Aspects of Reproduction: (a) mechanism of ova-implantation in the rat, (b) synthetic progestins and the menstrual cycle, (c) progesterone in pregnancy, (d) long-acting steroids in reproduction; (IV) Hormone Chemistry and Metabolism: (a) insulinase and diabetes mellitus, (b) glucagon; (V) Hormones and Stress: (a) endocrine changes following trauma in man, (b) adrenal influences upon the stomach.

The essays on serotonin, central nervous system (CNS), and metabolic processes, CNS and pituitary function, hormones and rhythms, binding of steroids to human plasma proteins, the whole section on reproduction, and the very scholarly treatise on endocrine changes following trauma are of outstanding interest.

Udenfriend and his associates conclude that serotonin is an important agent, but it remains to determine exactly where and how it functions. The isolation of both serotonin and norepinephrine in phylogenetically ancient areas of the brain suggests their involvement in the more fundamental functions of the brain. In a previous report, Brodie and Shore suggested that serotonin may be a transmitter substance involved in the stimulation of parasympathetic centers in subcortical areas, or that it may be involved in inhibition of sympathetic centers. Norepinephrine may then be the transmitter substance for central sympathetic impulses. Wooley and his associates also have suggested a possible role for serotonin in the brain. They observed that pulsations of oligodendroglia in tissue culture are enhanced by serotonin and inhibited by LSD. Consequently, Wooley's group suggested that the function of glial cells is to act as "stirring devices" to circulate the fluid in the central nervous system. The role of serotonin would then be one of activating glial motions. It will be recalled that Marrazi and Hart suggested that serotonin is a potent inhibitor of central synaptic transmission. Their studies on the transcallosal pathway of the cat revealed that serotonin may function normally as a humoral inhibitor. Only a few years have lapsed since the isolation and identification of serotonin, but the intense interest in this compound has advanced our knowledge of its biochemistry and pharmacology to such an extent that new leads concerning histamine, epinephrine, and norepinephrine have evolved.

Anderson's group reported on their numerous experiments and countless biochemical determinations on CNS and metabolic processes. They showed that transection of the midbrain in the dog induced profound changes in metabolic functions. These consisted of loss of nitrogen, decrease in glucose tolerance, and decrease in sensitivity to insulin, retention of sodium and

loss of potassium, creatinuria, and a rather abrupt decrease in urinary creatinine. In all of these biochemical aspects, functions returned to normal in about three weeks except for diminution of urinary creatinine which persisted. Renal clearance studies indicated an essentially normal glomerular filtration rate. Aldosterone secretion studied on one dog was not modified. When the lower cervical cord was transected, different effects were noted. As compared to the dogs with midbrain transections, nitrogen loss and cretinuria were more severe, sodium retention and potassium loss were less pronounced, and the decrease in urinary creatinine was delayed and gradual. The spinal dog showed alterations in carbohydrate metabolism opposite to that found in the midbrain dog. They postulated that some of these differences in the metabolic disturbances in the spinal and midbrain dogs may be related to alterations in adrenaline secretions—a decrease in adrenaline in the spinal dog and an increase in the midbrain dog. Their experiments on the hypothalamico-pituitary mechanism for the release of ACTH and TSH are of equal interest. They found that the so-called hypothalamico-pituitary mechanism for their release was disturbed by the transection of the midbrain. This procedure was not associated with an increase in ACTH secretion, but TSH secretion appeared to be depressed. After two weeks, TSH secretion appeared to be normal. In one dog remaining alive for one year after midbrain transection, the ACTH response to stress was definitely impaired. Transection of the spinal cord at the lower cervical level did not interfere with the ACTH release mechanism. Thus, it was concluded that it seemed reasonable to postulate the existence of a mesencephalic hypothalamico-pituitary activating system.

Greer's interpretation of his very interesting work on CNS and pituitary function was so cautious that it would be exceedingly difficult to take issue with him on any phase of his work. In his general discussion and conclusion he states that his concepts are "very tenuous and should not be taken as anything more than an individual flight of fancy." Nonetheless, it appears that the CNS can and does exert a definite influence over anterior pituitary function. The influence appears to be mediated through the hypothalamus, but the hypothalamus itself has not as yet been shown to be capable of independently regulating adenohypophyseal function without the contributions of the CNS. Greer presents experimental evidence indicating a "timing mechanism" in the pre-optic region of the hypothalamus for the control of cyclic gonadotropic activity in the female rat. This timing mechanism can be modified by the more posteriorly located part of the hypothalamus between the paraventricular nucleus and median eminence. Gonadotropic secretion is seemingly maintained by a "funneling" of influences to the pituitary through the median eminence; giving strength to this concept is the fact that the destruction of the median eminence, particularly in the male, results in gonadal atrophy. Thyrotropin (TSH) production seems controlled by an area located posteriorly and above the gonadotropin area. According to Greer, the primary area controlling ACTH release seems to be located somewhat ventrally and posterior to the TSH and "cyclic" gonadotropin centers and lies in the median eminence also. Greer concluded that (a) it seems unlikely that a single "neuro-

humor" can be responsible for controlling the different pituitary tropic secretions, (b) it seems much more plausible that separate substances must originate from the hypothalamus by way of the portal system, or possibly at times the general circulation, to stimulate the individual hormones from the anterior pituitary, and (c) although most investigators are in agreement that it is unlikely that a direct nervous influence reaches the pituitary to control its activity, it has not been established that such nervous impulses do not exist. In 1955 Guillemin, Rosenberg, and Hearn provided the most direct evidence for the existence of a hypothalamic neurohumoral substance.

Curt Richter's essay is by far one of the most thorough ever written on the subject of hormones and rhythms. He has amassed and revealed a superabundance of facts concerning behavioral rhythms in the rat and man since 16 May 1915, . . . "a fine day in Baltimore . . . one of those beautiful, clear days that should make everyone happy and glad to be alive." Richter's clinical description of Mrs. B. for that day, and the facts which led him to his present researches are revealed. This is a rare opportunity for one to do research on the researcher. His essay is a description of the various abnormal but regular cycles of behavior, mood, and metabolism that occur in psychiatric patients and patients with "periodic disease." These cycles range in length from one day to one and one-half years, each characterized by a normal and abnormal phase. The latter may last for years or a lifetime. While the etiology is not known, catatonic-schizophrenics with cycles of 20-40 days in length have been helped by thyroid medication. Similar cycles have been produced experimentally in rats by several forms of interference, e. g. thyroid, pituitary, or brain lesions. From his researches, two theories of the origin of the abnormally produced cycles were suggested: (a) based on the feed-back relationship between the small amounts of remaining functional thyroid tissue, the thyrotropic cells of the hypothalamus, the thyrotropic cells in the pituitary, and metabolic rate, and (b) based on the inclusion also of the follicle-stimulating and luteinizing hormone mechanisms of the pituitary. His experimentally produced cycles in rats closely resembled those cycles observed in patients with cyclic agranulocyctosis. Furthermore, some displayed a similarity of behavior and mood seen in catatonic-schizophrenic patients.

Sandberg, Slaunwhite, and Antoniades' essay on the binding of steroids to human plasma proteins is provocative, especially if one compares their data with those of Szego and Roberts. In brief, Sandberg and his associates seem somewhat convinced that (a) blood fractions IV-1, IV-4, and V are the major transporters of steroid hormones in the body, (b) steroid-protein binding involves free and conjugated steroids, (c) estrogens are the most strongly bound, corticosteroids the least, (d) the liver does not seem to play an important role in the evolution of a blood protein-steroid complex, although by itself it may form strongly bound steroid-protein complexes, and (e) in the case of certain steroids the erythrocytes are important agents in their transport in the body.

The section on reproduction reveals some new concepts. Rock, Garcia, and Pincus clinically evaluated the effects of 17 alpha-ethinyl-19-nortestos-

terone (I), 17 alpha-ethinyl-5 (10)-estraeneolone (II), and 17 alpha-ethyl-19-nortestosterone (III). All three compounds, by indices from temperature graphs, endometrial biopsies, and vaginal smears provided evidence indicating inhibition of corpus luteum development, and therefore probably ovulation, during the periods of treatment. Within five months following treatment with I and/or II, there were seven conceptions in a group of 38 patients.

Davis and Plotz's work reveals that large doses of progesterone are very effective in aiding women, with histories of repeated abortions, to give birth to living children. They also reported on the clinical efficacy of esterified and hydroxylated progesterone, 17 alpha hydroxy-progesterone-17N-caproate.

Junkman's report on long-acting steroids in reproduction will undoubtedly remain a classic now that most clinicians are resorting to the use of long-acting steroids in their practices. Much of the basic information regarding esterified estrogenic, and progestational steroids is presented in this essay. It is authentically documented, contains almost 75 per cent of his own findings, and is presented in a very readable manner.

The essay by Francis D. Moore on the endocrine changes following trauma in man is by far the most brilliant treatise ever presented on the subject. His passion for excellence is demonstrated not only in his literary style but in the careful documentation of his data presented in 49 figures. His theories are the result of many biochemical determinations on the surgical patient. In brief they may be summarized as follows: (a) severe tissue injury initiates post-traumatic metabolism in the presence of constant amounts of pituitary and adrenal hormones; these hormones are permissive, and the initiating substance may be a "wound hormone" from the wound itself; the neuro-endocrine activation of these hormones is essential in severe injury; (b) mild injury requires activation of the hypothalamic-pituitary-adrenal axis to initiate metabolic change; (c) in either case of mild or severe injury, with or without adrenal activation, the metabolic sequence subsequently "runs out" its course to the end of convalescence and long after its return to normal without further detectable adrenal steroid change.

Surgery has taken a new and more scientific place now that surgeons are concerned with the total body composition of the patient before surgery, during surgery, and during the period of convalescence. Moore's treatise should be highly welcomed by the student of surgery and metabolism.

In the final analysis, this volume is heavily laden with worthwhile information. Since this is the printed record of a conference, some effort should be made to reduce the cost of the volume. Perhaps the deletion of long reviews of the literature previously cited elsewhere may serve well to reduce the number of printed pages and thus reduce the cost of the volume. In this regard the Macy Foundation Symposia and the published annals of conferences of the New York Academy of Sciences represent an enviable job; furthermore, these volumes are approximately one-third the cost of the present series.

PHYSIOLOGY OF PREMATURITY. Transactions of the First Conference, March 21, 2, 23, 1956. Jonathan T. Lanman, Ed. New York, Josiah Macy, Jr., Foundation, 1957. 151 pp. $3.25.

The problem of prematurity has received a great deal of attention in recent years, and this book records a pertinent discussion by several prominent men in the field. The list of those who contributed is impressive, and it includes the names of Clement Smith, Jonathan Lanman, George Wislocki, Geoffrey S. Dawes, Alfred Jost, and many others. Circulation and the glands of internal secretion are discussed in relation to prematurity, but, for obvious reasons, only certain controversial aspects of these are taken up.

Fetal-maternal endocrinology in late pregnancy is considered in the first of two parts from the standpoint of those endocrine systems which tend to maintain and terminate pregnancy and those directed at maintaining homeostasis while the process goes on. The adrenals, the pituitary, their hormones, are among the many topics discussed and related to such things as hypoxia, fetal hemoglobin, liver glycogen, sodium excretion, the adrenogenital syndrome, and several additional subjects. An extremely interesting section concerns the prevention of prematurity in such people as diabetics, patients with placenta praevia, and those with dietary deficiencies.

The second part covers fetal and placental circulation in late pregnancy. Oxygen saturations, pressures and direction of flow, metabolic changes and response to anoxia, cardiac shunts, murmurs, and many other points are taken up. As in the first part, data derived from animal research are applied to situations in the human and speculations are made where indicated. In this regard the book is interesting and thought provoking.

The material is presented in dialogue form and no summary is given. Whether one likes this question-and-answer style or not is essentially a personal matter. The discussion does wander now and again, but this is not a serious defect because of the manner of presentation. Readers should be encouraged by this format to think further about the problems presented; the fairly extensive and pertinent bibliography at the end of each part is helpful for this purpose. The illustrations, tables, and printing are excellent and the over-all presentation clear. Finally, however, the book is primarily theoretical in nature, and for this reason would probably be of interest only to those who are well acquainted with the study of prematurity.

J. A. O'N.

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